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THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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VOLUME LXV
AUGUST TO DECEMBER, 1933

Smithsonian Institution
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National Museum

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AUGUST, 1933

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NATIONAL MUSEUM

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INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost.

Proof. Page proof will be furnished only upon special request. When cross-references are made in the text, the material referred to should be marked clearly on the galley proof in order that the proper page numbers may be supplied.

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THE MARINE BIOLOGICAL LABORATORY

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The Library Committee

C. E. McClung, *Chairman.*
Robert A. Budington.
E. E. Just.
M. M. Metcalf.
A. H. Sturtevant.

II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the

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President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is submitted my report as Treasurer of the Marine Biological Laboratory for the year 1932.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants, and a copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1932, the book value (cost) of the general Endowment Fund in the hands of the Central Hanover Bank and Trust Company as Trustee, was $906,415 in securities, and $311.49 in cash.

The book value (cost) of the Library Fund, was $195,949.19 in securities, and $127.56 in cash.

All of the income due during the year on the general Endowment Fund and on the Library Fund was collected with the exception of interest amounting to $791.00 on a mortgage belonging to the general Endowment Fund.

The Reserve Fund consisted of securities of the book value (cost) of $19,671.13 and cash of $1,623.59.

The Retirement Fund consisted of mortgages of the book value of $19,300 and cash of $2,413.62.

There has been no change in the investments of the other minor funds.

The land, buildings, equipment and library, excluding the Gansett and Devil's Lane tracts, represented an investment of $1,683,017.56, less depreciation of $327,455.68, or a net amount of $1,355,561.88.

Current expenses including $41,051.48 depreciation exceeded income by $24,585.55. Over $40,000 was expended from current funds in the acquisition of real property, alterations and additions to the electric system of the pumping station and Laboratory, on new equipment and on books, of which over $18,000 was expended for books.

At the end of the year the Laboratory owed $27,000 on bond and mortgage, and in accounts payable $8,921.41, most of which was a deferred liability to be paid one half in 1933 and the balance in 1934, and had $14,316.33 current funds in its bank accounts. Following is the balance sheet as of December 31, 1932, and the condensed statement of income and outgo for the year, also the surplus account.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1932

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank & Trust Company (of New York), Trustee—Schedules I-a and I-b		$1,102,803.24	
Securities and Cash—Minor Funds—Schedule II		10,698.88	$1,113,502.12
Plant Assets:			
Land—Schedule IV	$ 98,103.05		
Buildings—Schedule IV	1,224,618.42		
Equipment—Schedule IV	167,229.03		
Library—Schedule IV	193,067.06	$1,683,017.56	
Less Reserves for Depreciation		327,455.68	
		$1,355,561.88	
Securities and Cash in Reserve Fund		21,294.72	
Cash in Dormitory Building Fund		818.96	$1,377,675.56
Current Assets:			
Cash		$ 14,816.33	
Accounts—Receivable		10,449.87	
Inventories:			
Supply Department	$ 38,310.30		
Biological Bulletin	9,062.55	47,372.85	
Investments:			
Devil's Lane Property	$ 40,782.48		
Gansett Property	5,103.29		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	21,713.62	80,299.39	
Prepaid Insurance		3,540.16	
Items in Suspense (Net)		291.39	$ 156,769.99
			$2,647,947.67

Liabilities

Endowment Funds:		
General Endowment Funds—Schedule III	$1,102,803.24	
Minor Funds—Schedule III	10,698.88	$1,113,502.12
Plant Funds:		
Donations and Gifts—Schedule III	$1,029,372.61	
Other Investments in Plant from Gifts and Current Funds	346,302.95	
	$1,375,675.56	
Mortgage, Danchakoff Estate	2,000.00	$1,377,675.56
Current Liabilities and Surplus:		
Mortgage, Devil's Lane Property	$ 25,000.00	

Accounts—Payable:			
Current	$ 1,576.41		
Hixon Electric Company, Deferred	7,345.00	8,921.41	
Woods Hole Oceanographic Institution:			
Amounts received for Purchase of Books	$ 6,000.00		
Less Payments	5,457.45	542.55	
		$ 34,463.96	
Current Surplus—Exhibit C		122,306.03	$ 156.769.99
			$2,647,947.67

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1932

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 47,013.66		$ 47,013.66
Library Fund		8,655.26		8,655.26
Gifts		400.00		400.00
Instruction	8,417.64	9,930.00		1,512.36
Research	4,006.10	13,425.00		9,418.90
Evening Lectures	182.50		182.50	
Biological Bulletin and Membership Dues	8,485.33	9,929.71		1,444.38
Supply Department—Schedule V	46,644.84	43,944.55	2,700.29	
Mess—Schedule VI	24,211.48	26,821.84		2,610.36
Dormitories—Schedule VII	31,732.05	12,933.23	18,798.82	
(Interest and Depreciation charged to above Three Departments. See Schedules V, VI, and VII)	*36,026.61*			36.026.61
Dividends, General Biological Supply House, Inc.		2,032.00		2,032.00
Rents:				
Danchakoff Cottages	253.43	515.00		261.57
Microscopes		458.25		458.25
Garage, Railway, etc.		345.30		345.30
Newman Cottage	69.67	200.00		130.33
Janitor's House	34.61	375.00		340.39
Interest on Bank Balances		132.90		132.90
Sales of Duplicate Library Sets		26.38		26.38
Sundries		35.24		35.24
Maintenance of Plant:				
New Laboratory Expenses	21,850.49		21,850.49	
Chemical and Special Apparatus	10,141.22		10,141.22	

Maintenance, Buildings, and Grounds	9,073.44		9,073.44	
Library Department Expenses	8,864.80		8,864.80	
Carpenter Department Expenses	1,071.11		1,071.11	
Truck Expenses	970.53		970.53	
Sundry Expense	81.07		81.07	
Workmen's Compensation Insurance	624.01		624.01	
Pumping Station Expense	114.19		114.19	
General Expenses:				
Administration Expenses	15,137.10		15,137.10	
Endowment Fund Trustee	968.50		968.50	
Interest on Loans	100.00		100.00	
Bad Debts	321.16		321.16	
Mosquito Fund Contribution	100.00		100.00	
Naples Zoological Station	250.00		250.00	
Reserve for Depreciation	41,051.48		41,051.48	
Museum Expenses	3,028.73		3,028.73	
	$201.758.87	$177,173.32	$135,429.44	$110,843.89
Excess of Expenses over Income carried to Current Surplus—Exhibit C		24,585.55		24,585.55
	$201,758.87	$201.758.87	$135,429.44	$135,429.44

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT

YEAR ENDED DECEMBER 31, 1932

Balance, January 1, 1932		$141,346.02
Add:		
Reserve for Depreciation charged to Plant Funds		41,051.48
Original cost of 216,858 square feet of land in Gansett Tract remaining on hand January 1, 1932 set up		4,977.35
		$187,374.85
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV:		
Land—Kahler Estate	$ 1,000.00	
Buildings:		
Kahler Estate	7,266.57	
Alterations and Additions to Electric System of Pumping Station and Laboratory	8,553.00	
Other Items	1,244.71	
Equipment	4,263.55	
Library Books, etc.	18,024.49	
	$40,352.32	

Pensions and Allowances Paid	$1,040.00		
Less Income of Retirement Fund	909.05	130.95	
Excess of Expenses over Income for Year as shown in Exhibit B		24,585.55	65,068.82
Balance, December 31, 1932—Exhibit A			$122,306.03

Respectfully submitted,

LAWRASON RIGGS, JR.,

Treasurer.

V. THE REPORT OF THE LIBRARIAN

The sum of $24,000 again allowed by the Executive Committee for the year 1932 was budgeted by the Librarian in figures identical with the year before. Examination of the account books kept by the Librarian will show, however, that about $1,000 in each of the years 1931 and 1932 ($1,068.68 and $1,074.16 respectively) was not expended on the Library at the end of the year. The money was not readily available for Library purposes. The Laboratory has never charged against the Library carpentry work and expenditures for permanent equipment and has always coöperated with the Library in emergency purchases. Moreover, the number of "Biological Bulletin" subscriptions allowed the Library has increased in actual money value from $1,470.15 in 1926 to $4,233.34 in 1932, transferred to the Library in the form of current serial exchanges. When the end of the year came without outstanding bills to demand the complete sum, it seemed best to follow a procedure usual in business budgeting, and to start the new year without a surplus left over from the year before. The Librarian contributed somewhat to this surplus, also, by an economy in library personnel, allowing a position made vacant in the Fall of 1932 to remain unfilled. The vacancy will remain also through 1933.

The Woods Hole Oceanographic Institution appropriation of $1,000 for the year 1932–33 (March to March) added to the balance from their $5,000, appropriated in 1930, $1,431.99 in all, was expended as follows: Books (including old), $292.54; Current Serials, $318.64; Binding, $117.92; Back Sets, $702.89; Total, $1,431.99.

At the end of the year 1932, the Library contained 36,014 volumes and 76,275 reprints. Of the volumes, 5,601 are books and 30,413 bound back sets of serials. Of the reprints, 2,500 are bound and 73,775 in pamphlet form protected in boxes. The estimate of volumes is made direct from the accession stamp, that of the reprints from count. In the first report, that of 1919–20, containing a statement of the total holdings of the Library, the reprints, which had heretofore been entered in accession with the books and serials and in the same sequence, were segregated as pamphlets, and the accession number used only for a count of books and bound serial volumes. A round number, 5,000, was subtracted from the accession number to make the record of volumes correct. Since that time, we have not counted the reprints as among the volumes of the Library, not even in cases where they are bound, and many of them of good size, 200–500 pages. When the reprints that are bound, whether pamphlet size or book size, reach 5,000, the subtraction of 5,000 from our accessioned volumes can be cancelled and from that point on, all bound reprints be automatically counted by the accession stamp (an R1–R5,000 count is being made now in the reprints as they are being bound). It will be a great satisfaction to reach the 5,000 mark in bound reprints because from that point on the accession number will at any moment show the actual number of bound volumes in the Library. None-the-less, a separate count each year of the new books, serial binding, and reprint binding can be made automatically from our records. The record will be kept also of total reprints just as heretofore.

Twelve new serials bring the total current journals to 1,126: paid Marine Biological Laboratory subscriptions, 362; exchanges, 586; gifts, 147; paid Woods Hole Oceanographic Institution subscriptions, 31. Back sets of 86 serials were purchased: for the Marine Biological Laboratory, 48 were completed, and 31 partially filled in; for the Woods Hole Oceanographic Institution, 3 were completed, and 4 partially filled in. As we stated in the report for 1927, extensive hand-books and expeditions that continue to be issued serially, are included in their current issues in the count of current serials. Of the total number of current serials, 63 are of this nature in the Marine Biological Laboratory purchases, and 3 for the Woods Hole Oceanographic Institution. The new books added to the Library were 263; 201 (nearly all current) by the Marine Biological Laboratory and 62 by the Woods Hole Oceanographic Institution.

Dr. Metcalf's library presented by him to the Marine Biological Laboratory is the finest gift of books and reprints that the Library has ever received. During the past five years, he has sent to us 9,200 reprints, 277 books, and some very fine runs in serial back sets. This includes many publications in subjects other than Dr. Metcalf's special subjects of pelagic Tunicates and of Protozoa. Part of his card catalogue and the cases he gave with them are already in use in the reading-room and offices.

Miss Margaret Sumwalt presented 50 reprints to the Library when she left the Laboratory in the Fall. Another very acceptable gift was made through the coöperation of Dr. T. S. Palmer and Dr. Herbert

Friedmann. Ten volumes of " Auk " were presented that were needed to complete the second series of this journal. The Library is indebted to five authors who kindly presented books, and two publishers and authors in combined gift; from publishers, thirteen books were presented and we wish to acknowledge with thanks books sent by the following publishers:

P. Blakiston's Son & Company	2
Doubleday, Doran & Company, Inc.	1
Lea & Febiger	2
J. B. Lippincott Company	1
McGill University Press	1
McGraw-Hill Book Company	1
The Macmillan Company	2
Ray Long & Richard R. Smith, Inc.	2
W. B. Saunders Company	1

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-fifth session of the Marine Biological Laboratory for the year 1932.

1. *Attendance.* Reference to previous annual reports will show that the number of investigators in attendance at the Laboratory increased steadily from 176 in 1923 to a maximum of 362 in 1931—a gain of 106 per cent in 8 years. In 1932, for the first time since the World War, there was a sharp drop in the registration of investigators, which brought their number back to approximately that of 1928. This falling off in attendance is to be attributed chiefly, if not wholly, to the unfavorable financial conditions existing in most American universities which, though beginning somewhat earlier, became especially serious in 1932. It is to be noted that the decrease was proportionately much greater in the case of the younger beginning investigators and research assistants, where it amounted to approximately 20 per cent, than in that of the older so-called independent investigators in which it was only 10 per cent. This difference is what might have been expected from the fact that the enforced economies of the universities up to and including 1932 had affected most adversely the younger men. There are indications at the time of the writing of this report that university conditions in 1933 will be even worse than in 1932 and that a further decline in attendance in the class of independent investigators may therefore be anticipated. During the existence of this very real emergency it has been the policy of the Laboratory, to the extent permitted by its own resources, to reduce to a minimum the financial burdens of those of its investigators whose scientific work has already been handicapped by inadequate university support.

As contrasted with the registration of investigators, that of students in the courses in 1932 remained at its usual level, since although there was some reduction in the number of applicants, these still remained considerably in excess of the number of available places in most of the courses.

As in past years, the rise and fall of the attendance of investigators and research assistants by approximately 10-day intervals is shown in tabular form.

		1927	1928	1929	1930	1931	1932
May	30	7	15	9	6	6	8
June	10	50	64	55	50	51	54
"	20	114	140	139	153	153	127
"	30	212	240	197	208	217	172
July	10	247	281	238	253	258	225
"	20	247	282	242	250	273	245
"	30	245	272	249	253	281	248
August	10	234	250	256	254	302	257
"	20	208	226	243	245	280	236
"	30	168	183	220	204	239	190
September	10	110	112	157	122	136	129
"	20	50	43	59	44	69	58
"	30	12	14	14	8	14	13

2. *The Report of the Treasurer.* As anticipated in 1931, it has been impossible for the Laboratory to maintain its very favorable financial showing of that year. During 1932 two of its three chief sources of income have undergone marked reductions. These are first, the subscriptions received from Coöperating and Subscribing Institutions, which fell from $15,975.00 in 1931 to $11,625.00 in 1932. Knowing the serious financial problems that have had to be met by most of these institutions, the Laboratory acknowledges with especial gratitude the very generous degree of support that has been continued in spite of the present unfavorable conditions. Secondly, the loss in income from the sale by the Supply Department of biological material to schools and colleges has been of considerable magnitude. This loss is due partly to the decreased purchasing power of educational institutions and partly to the very severe competition from commercial firms, which under the present abnormal conditions are frequently willing to sacrifice fair and reasonable profits in order to be able to continue their business at all. The third chief source of revenue of the Laboratory, namely, the income received from investments, showed no great falling-off in 1932; but certain unavoidable losses from this source are anticipated in 1933. Though, on the whole, the Laboratory has fared much better during the present business depression than most other educational and scientific institutions, the need of the strictest economy has been felt, and in

the budget for 1933 approved by the Executive Committee late in the summer of 1932, and subsequently several times revised, every effort has been made to keep the expenditures of the Laboratory within its income. While the carrying out of this policy may result in some minor inconveniences to both investigators and students, it is believed that neither research nor instruction will be seriously handicapped unless conditions should become considerably worse than they are at the time of the preparation of this report.

3. *The Report of the Librarian.* The progress of the Library during the past year, especially in its relation to that of previous years, may perhaps best be shown in the tabular form adopted in previous reports.

	1925	1926	1927	1928	1929	1930	1931	1932
Serials received currently	500	628	764	874	985	1060	1080	1126
Total number of bound volumes	15000	18200	22800	26500	28300	31500	33800	36000
Reprints	25000	38000	43000	51000	59000	64000	70000	76000

Additional grateful acknowledgment is here made of the very generous gift of Dr. M. M. Metcalf mentioned above in the report of Librarian.

4. *Purchases of Property.* In March, 1932, a valuable addition to the land owned by the Laboratory was made by the purchase of the Kahler property (approximately 45 by 79 feet) facing the Eel Pond and lying between the New Dormitory and the Hubbard property acquired in 1924. The holdings of the Laboratory on East Street are now unbroken and the possibilities for the advantageous erection of new buildings in the future are greatly increased. For the present the house already on this property is being used as a men's dormitory.

5. *Changes in the Electrical System.* During the spring of 1932 important changes were made in the electrical system of the Laboratory, at a cost which, spread over a period of three years, will amount to slightly less than $15,000.00. These changes had as their chief objects: (1) increasing the capacity and flexibility of the system and in particular providing an adequate source of alternating current for the operation of the enlarged X-ray plant, etc., (2) prevention of the rapid deterioration of the storage battery, which with the growth of the Laboratory had become seriously overloaded, (3) securing a constant voltage for certain types of scientific work which were difficult or impossible to carry on with the existing arrangements, (4) provision for the operation of the salt water pumps at all hours, thereby eliminating the serious shortages of sea water which with the growth of the Laboratory were occurring with increasing frequency, (5) securing a lower cost of opera-

tion of the plant. The experience of the past summer indicates that all of these objects have been attained, and the thanks of the Laboratory are due to Dr. Pond who, with the able assistance of Mr. Larkin, so successfully handled the various complicated problems that arose in connection with this work.

6. *Technical Manager.* With the increasing complexity of the methods and apparatus used in biological work, the need has become urgent for a person who can devote a larger amount of his time to the design and care of special apparatus and to other technical needs of the Laboratory and its investigators than one in residence merely during the summer months. The Laboratory has been fortunate in securing in this capacity, with the title of Technical Manager, Dr. S. E. Pond who for 7 years has served as Custodian of Apparatus, and whose experience in this special field is almost unique. Dr. Pond will be in permanent residence in Woods Hole, where, in addition to his duties in connection with the various technical problems of the Laboratory relating to research, he will continue his own investigations in the field of calcification. This important addition to the staff of the Laboratory will do much not only to facilitate the work of its investigators during the regular summer season, but to make available its special facilities to properly qualified workers at other times of the year.

7. *Lectures and Scientific Meetings.* During the summer of 1932 there were given 10 regular evening lectures in addition to 54 shorter scientific papers in which recently completed work was reported by investigators. As in 1931, a special all-day scientific session near the end of the season, devoted primarily to work accomplished during the summer, was most successful. The Spaeth Memorial Lecture for 1932 was delivered by Professor Richard Goldschmidt of Berlin-Dahlem.

8. *Board of Trustees.* During 1932 three Trustees of long standing, Dr. H. C. Bumpus, Dr. R. A. Harper, and Dr. M. M. Metcalf, were honored by being elected Trustees Emeritus by the Corporation. The vacancies so created were filled at the same meeting by the election of the following new Trustees: Dr. W. C. Allee (Class of 1935), Dr. H. B. Bigelow (Class of 1936), and Dr. Franz Schrader (Class of 1934). It is with deep regret that record is here made of the death of Dr. William Patten of Dartmouth College, who for many years rendered valuable services to the Laboratory as a Trustee and otherwise, and who in 1930 became a Trustee Emeritus.

There are appended as parts of this report:

1. The Staff, 1932.
2. Investigators and Students, 1932.
3. A Tabular View of Attendance, 1928–1932.

4. Subscribing and Coöperating Institutions, 1932.
5. Evening Lectures, 1932.
6. Shorter Scientific Papers, 1932.
7. Members of the Corporation, August, 1932.

Respectfully submitted,

M. H. Jacobs,
Director.

1. THE STAFF, 1932

Merkel H. Jacobs, *Director,* Professor of General Physiology, University of Pennsylvania.

Associate Director: ——

ZOÖLOGY

I. Investigation

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
E. G. Conklin, Professor of Zoölogy, Princeton University.
Caswell Grave, Professor of Zoölogy, Washington University.
H. S. Jennings, Professor of Zoölogy, Johns Hopkins University.
Frank R. Lillie, Professor of Embryology, the University of Chicago.
C. E. McClung, Professor of Zoölogy, University of Pennsylvania.
S. O. Mast, Professor of Zoölogy, Johns Hopkins University.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoölogy, Harvard University.
E. B. Wilson, Professor of Zoölogy, Columbia University.
Lorande L. Woodruff, Professor of Protozoölogy, Yale University.

II. Instruction

T. H. Bissonnette, Professor of Biology, Trinity College.
E. C. Cole, Professor of Biology, Williams College.
B. R. Coonfield, Instructor in Biology, Brooklyn College.
O. E. Nelsen, Instructor in Zoölogy, University of Pennsylvania.
A. W. Pollister, Instructor in Zoölogy, Columbia University.
L. P. Sayles, Instructor in Biology, College of the City of New York.
A. E. Severinghaus, Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University.

Junior Instructors

C. E. Hadley, Assistant Professor of Biology, New Jersey State Teachers College at Montclair.
S. A. Matthews, Associate in Anatomy, School of Medicine, University of Pennsylvania.

PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
Rachel Bowling, Instructor in Zoölogy, Columbia University.
Robert W. Stabler, Instructor in Zoölogy, University of Pennsylvania.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

L. G. Barth, Instructor of Experimental Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.

PHYSIOLOGY

I. Investigation

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, the University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

William R. Amberson, Professor of Physiology, University of Tennessee.
Philip Bard, Assistant Professor of Physiology, Harvard Medical School.
Ralph W. Gerard, Assistant Professor of Physiology, the University of Chicago.
Laurence Irving, Associate Professor of Physiology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
Margaret Sumwalt, Assistant Professor of Physiology, Woman's Medical College of Pennsylvania.

Special Lecturers

Edwin J. Cohn, Associate Professor of Physical Chemistry, Harvard University.
Henry J. Fry, Associate Professor of Biology, Washington Square College New York University.

E. Newton Harvey, Professor of Physiology, Princeton University.
Selig Hecht, Professor of Biophysics, Columbia University.
Merkel H. Jacobs, Professor of General Physiology, University of Pennsylvania.
Balduin Lucké, Associate Professor of Pathology, University of Pennsylvania.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Pennsylvania.
James P. Poole, Professor of Evolution, Dartmouth College.
G. W. Prescott, Assistant Professor of Biology, Albion College.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Hazel Blanchard, Doris Endrejat, Mary A. Rohan, Assistants.

DEPARTMENT OF EXPERIMENTAL RADIOLOGY

G. Failla, Physicist, Memorial Hospital, New York City.

CHEMICAL SUPPLIES

Oscar W. Richards, Instructor in Biology, Yale University.

SCIENTIFIC APPARATUS AND TECHNICAL SUPPLIES

Samuel E. Pond, Assistant Professor of Physiology, Schools of Medicine and Dentistry, University of Pennsylvania, in charge.
A. R. Apgar, Photographer.
J. D. Graham, Glassblower.
Lester F. Boss, Mechanician.
P. H. Liljestrand, Assistant.

MUSEUM

George M. Gray, Curator.

SUPPLY DEPARTMENT

James McInnis, Manager.
A. M. Hilton, Collector.
Milton B. Gray, Collector.
Walter Kahler, Collector.
Geoffrey Lehy, Collector.
A. W. Leathers, Shipping.

BOATS

John J. Veeder, Captain. E. M. Lewis, Chief Engineer.

F. M. MacNaught, Business Manager.
Thomas Larkin, Superintendent of Mechanical Department.
William Hemenway, Carpenter.

2. INVESTIGATORS AND STUDENTS, 1932

Independent Investigators

Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania, School of Medicine.
Amberson, William R., Professor of Physiology, University of Tennessee.
Anderson, Rubert S., Research Associate, Princeton University.
Armstrong, Philip B., Assistant Professor of Anatomy, Cornell University Medical College.
Baitsell, George A., Professor of Biology, Yale University.
Baker, Horace B., Associate Professor, University of Pennsylvania.
Ballard, William W., Instructor in Zoölogy, Dartmouth College.
Bard, Philip, Assistant Professor of Physiology, Harvard Medical School.
Barth, L. G., Instructor, Columbia University.
Beams, H. W., Assistant Professor of Zoölogy, State University of Iowa.
Belkin, Morris, Instructor, New York University.
Bigelow, Robert P., Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.
Bissonnette, T. Hume, Professor of Biology, Trinity College.
Bodansky, Aaron, Research Biochemist, Hospital for Joint Diseases, New York.
Borodin, Dmitry N., Yonkers, New York.
Bowling, Rachel, Instructor in Zoölogy, Columbia University.
Boyer, Donald A., Instructor, the University of Chicago.
Bozler, Emil, Fellow in Medical Physics, Johnson Foundation for Medical Physics.
Bridges, Calvin B., Research Assistant, Carnegie Institution of Washington.
Brinley, Floyd J., Assistant Professor of Zoölogy, North Dakota State College.
Brooks, M. M., Research Associate, University of California.
Brooks, S. C., Professor of Zoölogy, University of California.
Brown, Dugold E. S., Instructor in Physiology, New York University.
Buchsbaum, Ralph, Instructor, the University of Chicago.
Budington, Robert A., Professor of Zoölogy, Oberlin College.
Cable, Raymond M., Teaching Fellow, New York University.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Callow, Bessie R., In Charge of Rheumatic Fever Laboratory, New York University, Medical College.
Carothers, E. Eleanor, Lecturer in Zoölogy, University of Pennsylvania.
Carpenter, Russell L., Associate in Anatomy, College of Physicians and Surgeons, Columbia University.
Castle, William A., Instructor in Biology, Brown University.
Cattell, Ware, Garrison, New York.
Chambers, Robert, Research Professor, Washington Square College, New York University.
Chanutin, Alfred, Professor of Biochemistry, University of Virginia.
Cheney, Ralph H., Chairman, Biology Department, Long Island University.
Chidester, F. E., Professor of Zoölogy, West Virginia University.

Clark, Eleanor Linton, University of Pennsylvania.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania.
Clowes, George H. A., Director of Research, Lilly Research Laboratories.
Coe, Wesley R., Professor of Biology, Yale University.
Cohen, Rose S., Graduate Assistant in Zoölogy, University of Cincinnati.
Cole, Elbert C., Associate Professor of Biology, Williams College.
Cole, Kenneth S., Assistant Professor of Physiology, Columbia University.
Cole, Robert H., Oberlin College.
Conklin, Edwin G., Professor of Biology, Princeton University.
Coonfield, B. R., Instructor in Biology, Brooklyn College.
Copeland, Manton, Professor of Biology, Bowdoin College.
Cowles, R. P., Professor of Zoölogy, Johns Hopkins University.
Croasdale, Hannah T., Assistant in Botany, University of Pennsylvania.
Darlington, C. D., Cytologist, John Innes Horticultural Institution, England.
Dawson, Alden B., Associate Professor of Zoölogy, Harvard University.
Donaldson, Henry H., Member, Wistar Institute.
Dreyer, William A., Instructor in Zoölogy, University of Cincinnati.
Duryee, William R., Instructor, Department of Zoölogy, Northwestern University.
Edwards, Dayton J., Associate Professor of Physiology, Cornell University Medical College.
Einarson, Larus, Research Fellow of the Rockefeller Foundation, Johns Hopkins University Medical School.
Fleisher, Moyer S., Professor of Bacteriology and Hygiene, St. Louis University, School of Medicine.
Fraser, Doris A., Assistant in Anatomy, University of Pennsylvania, Medical School.
Fry, Henry J., Professor of Biology, Washington Square College, New York University.
Garrey, W. E., Professor of Physiology, Vanderbilt University School of Medicine.
Gerard, R. W., Associate Professor of Physiology, the University of Chicago.
Glaser, O. C., Professor, Amherst College.
Glaser, R. W., Associate Member, Rockefeller Institute for Medical Research.
Goldforb, A. J., Professor of Biology, College of the City of New York.
Goldschmidt, Richard B., Director, Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem, Germany.
Goldsmith, E. D., Assistant in Zoölogy, Harvard University.
Goodrich, H. B., Professor of Biology, Wesleyan University.
Gould, R. Gordon, Jr., Tutor in Bio-chemical Sciences, Harvard University.
Grave, B. H., Professor of Zoölogy, DePauw University.
Grave, Caswell, Professor of Zoölogy, Washington University.
Green, David E., Assistant, Washington Square College.
Guerlac, Henry E., Assistant in Physiology, Cornell University.
Hadley, Charles E., Assistant Professor of Biology, New Jersey State Teachers College at Montclair.
Hahnert, William F., National Research Fellow in Biological Sciences, Johns Hopkins University.
Harnly, Morris H., Assistant Professor, Washington Square College, New York University.
Harvey, Ethel B., Research Investigator, Princeton University.
Harvey, E. N., Professor of Physiology, Princeton University.
Heilbrunn, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
Henshaw, P. S., Biophysicist, Memorial Hospital, New York City.
Herrick, Earl H., Professor of Biology and Head of Department, Louisiana State Normal College.
Hill, Samuel E., Assistant in General Physiology, Rockefeller Institute.
Hoadley, Leigh, Professor of Zoölogy, Harvard University.

Hodge, Charles, Jr., Instructor in Zoölogy, University of Pennsylvania.
Hook, Sabra J., Assistant Professor of Biology, University of Rochester.
Hoppe, Ella N., Research Assistant in Biology, New York State Department of Health.
Howe, H. E., Editor, Industrial and Engineering Chemistry.
Howland, Ruth B., Associate Professor of Biology, Washington Square College, New York University.
Huettner, Alfred F., Professor of Biology, Washington Square College, New York University.
Hull, Frank M., Head of Department of Biology, University of Mississippi.
Hunt, Thomas E., Assistant Professor of Anatomy, University of Alabama, School of Medicine.
Irving, Laurence, Associate Professor of Physiology, University of Toronto.
Jackson, J. R., Graduate Assistant in Botany, University of Missouri.
Jacobs, Merkel H., Professor of General Physiology, University of Pennsylvania.
Jahn, Theo. L., Fellow, National Research Council.
Jenkins, George B., Professor and Director Department of Anatomy, George Washington University.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt University, School of Medicine.
Johnston, Janet L., 250 S. 21st Street, Philadelphia, Pennsylvania.
Kaufmann, Berwind P., Professor of Botany, University of Alabama.
Keil, Elsa M., Instructor in Zoölogy, New Jersey College for Women.
Kerr, Thomas, National Research Fellow, University of Pennsylvania.
Keyes, D. B., Professor of Industrial Chemistry, University of Illinois.
Kidder, George W., Tutor, College of the City of New York.
Kirkpatrick, T. Bruce, Associate Professor of Physical Education, Columbia University.
Klein, Henry, Research Fellow, Johns Hopkins University.
Knower, Henry McE., Associate Professor of Anatomy, Albany Medical College.
Knowlton, Frank P., Professor of Physiology, Syracuse University, College of Medicine.
Krieg, Wendell J. S., Instructor in Anatomy, New York University.
Lacaillade, Charles Wm., Fellow, The Rockefeller Institute.
Lackey, James B., Professor of Biology, Southwestern.
Lancefield, Donald E., Associate Professor of Zoölogy, Columbia University.
Lancefield, Rebecca C., Associate in Bacteriology, Rockefeller Institute.
Laug, Edwin P., Instructor in Physiology, University of Pennsylvania.
Levy, Milton, Instructor, University and Bellevue Hospital Medical College.
Lewis, Ivey F., Professor of Botany, University of Virginia.
Lillie, Frank R., Chairman of the Department of Zoölogy, the University of Chicago.
Lillie, Ralph S., Professor of General Physiology, the University of Chicago.
Linderstrom-Lang, Kaj. M., Inspector, Carlsberg Laboratorium.
Lipmann, Fritz, Associate, Rockefeller Foundation.
Loefer, John B., University Fellow, University College, New York University.
Lynch, Ruth Stocking, Instructor, Johns Hopkins University.
McGoun, Ralph C., Jr., Instructor in Biology, Amherst College.
McGregor, James H., Professor of Zoölogy, Columbia University.
Machlis, Samuel, Assistant in Biology, Washington Square College, New York University.
Magruder, Samuel R., Graduate Assistant in Zoölogy, University of Cincinnati.
Martin, Earl A., Chairman of Biology Department, Brooklyn College.
Mast, S. O., Professor of Zoölogy, Johns Hopkins University.
Mathews, Albert P., Carnegie Professor of Biochemistry and Head of Department, University of Cincinnati.

Matthews, Samuel A., Associate in Anatomy, University of Pennsylvania.
Mazia, Daniel, Undergraduate Student, University of Pennsylvania.
Michaelis, Leonor, Member, Rockefeller Institute for Medical Research.
Miller, Forrest W., Graduate Assistant, University of Pittsburgh.
Montalenti, Giuseppe, Assistant in the Zoölogical Institute, Rome University, Rome, Italy.
Moore, A. R., Research Professor of Physiology, University of Oregon.
Morgan, Lilian V., California Institute of Technology.
Morgan, T. H., Professor of Biology, California Institute of Technology.
Morrill, Charles V., Associate Professor of Anatomy, Cornell University Medical College.
Nelsen, Olin E., Instructor in Zoölogy, University of Pennsylvania.
Nelson, E. Cliff, Student Assistant, Johns Hopkins School of Hygiene and Public Health.
Nonidez, José F., Assistant Professor of Anatomy, Cornell University Medical College.
Orias, Oscar, Fellow, Rockefeller Foundation.
Osterhout, W. J. V., Member, Rockefeller Institute for Medical Research.
Packard, Charles, Assistant Professor of Zoölogy, Columbia University.
Palmer, Albert H., New York University Medical College.
Papenfuss, G. F., Graduate Student, Johns Hopkins University.
Parker, G. H., Professor of Zoölogy, Harvard University.
Parpart, Arthur K., Instructor in Physiology, Princeton University.
Payne, Fernandus, Professor of Zoölogy, Indiana University.
Plough, Harold H., Professor of Biology, Amherst College.
Pollister, Arthur W., Instructor in Zoölogy, Columbia University.
Pollister, Priscilla Frew, Graduate Student, Columbia University.
Pond, Samuel E., Assistant Professor of Physiology, University of Pennsylvania, Medical School.
Poole, James P., Professor of Evolution, Dartmouth College.
Potter, Truman S., Seymour Coman Fellow in Preventive Medicine, University of Chicago.
Prescott, G. W., Assistant Professor of Biology, Albion College.
de Renyi, George S., Associate Professor of Anatomy, University of Pennsylvania.
Reznikoff, Paul, Instructor in Medicine, Cornell University Medical College.
Richards, Oscar W., Instructor in Biology, Yale University.
Robbins, William J., Professor of Botany, University of Missouri.
Robert, Nan L., Instructor in Zoölogy, Hunter College.
Root, Walter S., Associate Professor of Physiology, College of Medicine, Syracuse University.
Rugh, Roberts, Instructor in Zoölogy, Hunter College.
Saeger, Albert C., National Research Fellow in the Biological Sciences, National Research Council.
Sayles, Leonard P., Instructor in Biology, College of the City of New York.
Schauffler, William G., Princeton, New Jersey.
Scherp, Henry W., Assistant, Rockefeller Institute for Medical Research.
Schmidt, Ida T. Genther, Junior Research Fellow, Research Foundation of the Children's Hospital, Cincinnati.
Schmidt, Leon H., Research Fellow, University of Cincinnati, College of Medicine.
Schmieder, Rudolf G., Instructor in Zoölogy, University of Pennsylvania.
Schopper, E. Werner, Pathological Institute, Giessen, Germany.
Schrader, Franz, Professor of Zoölogy, Columbia University.
Schrader, Sally Hughes, Instructor in Zoölogy, Sarah Lawrence College.
Scott, Sister Florence Marie, Assistant Professor, Seton Hill College.
Severinghaus, Aura E., Assistant Professor of Anatomy, Columbia University.
Shumway, Waldo, Professor of Zoölogy, University of Illinois.

Silvey, J. K. Gwynn, Instructor, University of Michigan.
Sonneborn, Tracy M., Research Associate, Johns Hopkins University.
Speicher, B. R., Graduate Assistant, University of Pittsburgh.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stabler, Robert M., Instructor in Zoölogy, University of Pennsylvania.
Stanley, Wendell M., Research Assistant, Rockefeller Institute for Medical Research.
Steinbach, Henry Burr, Instructor in Zoölogy, University of Pennsylvania.
Stewart, Dorothy R., Assistant Professor of Biology, Skidmore College.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.
Stokey, Alma G., Professor of Botany, Mount Holyoke College.
Street, Sibyl F., Graduate Student, the University of Chicago.
Sturtevant, A. H., Professor of Genetics, California Institute of Technology.
Sumwalt, Margaret, Assistant Instructor, University of Pennsylvania, Medical School.
Sun, Tson P., Student, University of Pennsylvania.
Tang, Pei-Sung, Instructor in General Physiology, Harvard University.
Tashiro, Shiro, Professor of Biochemistry, University of Cincinnati, College of Medicine.
Taylor, G. Wellford, Fellow in Biology, Princeton University.
Taylor, W. R., Professor of Botany, University of Michigan.
Titus, Charles P., East Orange, New Jersey.
Vicari, Emilia M., Associate in Anatomy, Cornell University Medical College.
Warren, Howard C., Stuart Professor of Psychology, Princeton University.
Weisman, Maxwell N., Graduate, Columbia University.
Weiss, Paul A., Sterling Research Fellow, Research Institute of the Academy of Science in Vienna, Austria.
Wenrich, D. H., Professor of Zoölogy, University of Pennsylvania.
Whedon, Arthur D., Professor of Zoölogy and Head of Department, North Dakota State College.
Whiting, Anna R., Professor of Biology, Head of Department, Pennsylvania College for Women.
Whiting, P. W., Professor of Zoölogy, University of Pittsburgh.
Wieman, H. L., Professor of Zoölogy, University of Cincinnati.
Willier, Benjamin H., Professor of Zoölogy, the University of Chicago.
Wilson, Edmund B., Professor Emeritus, Columbia University.
Winsor, Agnes A., Volunteer Associate, Johns Hopkins University.
Winsor, Charles P., Associate in Biology, Johns Hopkins University.
Witschi, Emil, Professor of Zoölogy, State University of Iowa.
Wolf, E. Alfred, Associate Professor of Zoölogy, University of Pittsburgh.
Woodruff, Lorande Loss, Professor of Protozoölogy, Yale University.
Young, Roger Arliner, Assistant Professor of Zoölogy, Howard University.
Zeleny, Charles, Professor of Zoölogy, University of Illinois.
Zirkle, Conway, Associate Professor, University of Pennsylvania.

Beginning Investigators

Ashley, Alta, Research Assistant, University of Cincinnati and Children's Hospital Research Foundation.
Atlas, Meyer, Assistant in Zoölogy, Columbia University.
Beltran, Enrique, Professor of Zoölogy, University of Mexico.
Bradbury, Hester Ann, Hardwick, Massachusetts.
Bridges, John C., Graduate Student, Morehouse College.
Burr, Edith Rogers, Assistant in Zoölogy, Barnard College.
Butler, Thomas, Student, Vanderbilt Medical School.
Campbell, Dan H., Laboratory Assistant, Washington University.
Carlson, J. Gordon, Instructor in Biology, Bryn Mawr College.

Carson, Rachel L., Graduate Student, Johns Hopkins University.
Chao, Iping, Graduate Student, the University of Chicago.
Chen, T. T., Instructor, University of Pennsylvania.
Child, George P., Assistant in Biology, New York University.
Church, Charles F., Assistant Instructor in Pediatrics, University of Pennsylvania Medical School.
Clark, Jean M., Graduate Student, University of Pennsylvania.
Corson, Samuel A., Research Assistant, Graduate Student, University of Pennsylvania.
Costello, Donald P., Instructor in Zoölogy, University of Pennsylvania.
Crummy, Pressley L., Graduate Assistant, University of Pittsburgh.
Dan, Katsuma, Graduate Student, University of Pennsylvania.
Dieter, Clarence D., Assistant Professor of Biology, Washington and Jefferson College.
Eastlick, Herbert L., Graduate Assistant in Zoölogy, Washington University.
Fennell, Richard A., Assistant Professor, Birmingham College.
Fish, Harold S., Student and Assistant in Zoölogy, Harvard University.
Fuchs, W. Barrett, Student Assistant in Biology, American University.
Grand, C. G., Research Assistant, Washington Square College, New York University.
Henshaw, Christine T., Assistant Biophysicist, Memorial Hospital, New York City.
Hitchcock, Harold B., Assistant, Williams College.
Hodge, Ruth Patrick, Graduate Student, University of Virginia.
Kaliss, Nathan, Assistant in Zoölogy, Columbia University.
Kanrich, Dorothy M., Graduate Student in Physiology and Instructor, Carnegie Institute of Technology, Pittsburgh, Pennsylvania.
Keltch, Anna K., Eli Lilly Research Laboratory.
Kempton, Rudolf T., Instructor, New York University.
Keosian, John, Assistant in Biology, Washington Square College, New York University.
Kiess, Mary D., University of Pennsylvania.
Kille, Frank R., Graduate Student, University of Chicago.
Kinney, Elizabeth T., Lecturer in Zoölogy, Barnard College.
Landowne, Milton, Fellow in Biology, College of the City of New York.
Lawlor, Sister Anna Catherine, Instructor in Biology, College of Saint Elizabeth.
L'Heritier, Philippe, Rockefeller Fellow, École Normale Supérieure, Paris, France.
Lundstrom, Helen M., Chemist, Children's Hospital of Philadelphia.
Marsland, Douglas A., Assistant Professor of Biology, Washington Square College, New York University.
Metzner, Jerome, Graduate Student, Columbia University.
Mihalik, Peter, Assistant Professor, University of Budapest.
Modell, Walter, Assistant in Anatomy, Cornell University Medical College.
Morris, John E., Graduate Student and Laboratory Assistant, Fisk University.
Moseley, John E., Student of Biology, Harvard College.
Nicoll, Paul A., Assistant in Zoölogy, Washington University.
Ormsby, Andrew A., Research Assistant, Pennsylvania State College.
Pandit, C. G., Assistant Director, King Institute of Preventive Medicine, Madras, India.
Pierce, Madelene E., Instructor in Zoölogy, Vassar College.
Pierson, Bernice F., Graduate Student, Johns Hopkins University.
Prosser, C. Ladd, Graduate Student, Johns Hopkins University.
Rowland, Claude R., Assistant in Zoölogy, Columbia University
Rundles, Wayne, Undergraduate Assistant, DePauw University.

Russell, Henry D., Student, Harvard University.
Schechter, Victor, Tutor in Department of Biology, College of the City of New York.
Scott, Allan C., Assistant in Zoölogy, Columbia University.
Shapiro, Herbert, Assistant in Biology, Princeton University.
Sichel, Ferdinand J. M., Assistant in Biology, Washington Square College, New York University.
Smith, Bruce A., Graduate Student, University of Pittsburgh.
Smith, Emil L., Assistant in Zoölogy, Columbia University.
Southwick, Walter E., Austin Teaching Fellow, Harvard University.
Specht, Heinz, Graduate Student, Johns Hopkins University.
Sturdivant, Harwell P., Assistant Professor of Biology, Emory University.
Summers, Francis M., Columbia University.
Taylor, Georgiana, Graduate Student in Zoölogy, University of Pittsburgh.
TeWinkel, Lois E., Graduate Student, Columbia University.
Thomas, Thurlo B., Graduate Assistant, Oberlin College.
Tittler, Irving A., Assistant in Zoölogy, Columbia University.
Townsend, Grace, Fellow, the University of Chicago.
Walker, Paul A., Graduate Student, Harvard University.
Wilson, Hildegard, Teaching Fellow, University and Bellevue Hospital, New York University, Medical College.
Winokur, Morris, Fellow, College of the City of New York.

Research Assistants

Alderman, Evangeline, Instructor, Wellesley College.
Beck, Lyle V., Graduate Assistant, Department of Zoölogy, University of Pittsburgh.
Butt, Charles, Research Assistant, Princeton University.
Chute, A. Laurence, Fellow in Physiology, University of Toronto.
Crampton, Clair B., Instructor, Wesleyan University.
Doyle, William L., Assistant in Zoölogy, Johns Hopkins University.
Driggs, Marshall F., Medical Student, Cornell University Medical College.
Dunn, Edwin E., Research Assistant, University of Cincinnati.
Eyre, Sarah W., Research Assistant, Long Island University.
Gilmore, Kathryn, Technical Assistant, University of Pittsburgh.
Goodson, Mary L., Student, Barnard College.
Graham, C. H., Research Assistant, University of Pennsylvania.
Harnly, Marie L., Assistant in Biology, Washington Square College, New York University.
Harryman, Ilene, Lilly Research Laboratories.
Hill, Edgar S., Research Assistant, Rockefeller Institute.
Michaelis, Eva, Research Assistant, Columbia University.
Mills, Sylvia M., Research Assistant, Radcliffe College.
Moreland, Ferrin B., Graduate Assistant, Vanderbilt University.
Pace, Donald M., Research Assistant, General Physiology, Johns Hopkins University.
Parks, Elizabeth K., Instructor in Histology and Embryology, Boston University School of Medicine.
Parks, Mark E., Assistant Instructor in Biology, New York University.
Porter, Helen, Research Assistant, Harvard University.
Rawles, Mary E., Research Assistant, the University of Chicago.
Robertson, C. W., Assistant in Biology, New York University.
Sanger, Grant, Medical Student, Cornell University Medical College.
Sell, James P., Laboratory Assistant, Yale University.
Starkey, William F., Graduate Student in Zoölogy, University of Pittsburgh.
Wade, Lucille W., Eli Lilly Research Laboratories.
Young, Saul B., Technician, Rockefeller Institute for Medical Research.

Students

BOTANY

Bach, Doris A., University of Michigan.
Brubaker, Ethel, Teacher of Biology, Philadelphia High School.
Gustafson, Alton H., Instructor in Biology, Williams College.
Lawlor, John T., Jr., Austin Teaching Fellow, Harvard University.
MacArthur, Mary, Student, Acadia University.
Marquette, William G., Student, Columbia University.
Primrose, Helen L., Hunter College.
Russell, Dorothy M., Pennsylvania College for Women.
Spangler, Elizabeth A., Wheaton College.
Strongman, Louise E., Student, Radcliffe College.
Webster, Margaret D., Dalhousie University.
Weintraub, Robert L., George Washington University.
Wilhelm, Helen M., Hunter College.
Wismer, Virginia, Assistant in Botany, University of Pennsylvania.

EMBRYOLOGY

Aiken, Robert B., Research Fellow, University of Vermont.
Belcher, Jane C., Colby College.
Bradbury, Hester A., Hardwick, Massachusetts.
Burrows, Robert B., Jr., Assistant Graduate Student, Yale University.
Cowles, Janet M., Johns Hopkins University.
Cummings, Frances, 122 Elm Street, Meriden, Connecticut.
Dee, John E., Wesleyan University.
Dieter, Clarence D., Assistant Professor of Biology, Washington and Jefferson College.
Eastlick, Herbert L., Graduate Assistant in Zoölogy, Washington University.
Heiss, Mary E., Wellesley College.
Hoover, Margaret E., Student, Smith College.
Hussey, Kathleen L., Fellow in Zoölogy, Ohio Wesleyan University.
Kleinholz, Lewis H. K., Instructor in Anatomy, Colby College.
Kugler, Otto E., Tutor, Illinois University.
Lewis, Ralph H., Assistant in Biology, University of Rochester.
Miller, Dorothy K., Bryn Mawr College.
Morris, John E., Laboratory Assistant in Biology, Fisk University.
Olsen, Marlow W., Junior Poultry Biologist, United States Department of Agriculture.
Penn, Amos B. K. C., Graduate Student, Johns Hopkins University.
Pfeifer, Katherine, Teacher at Soldan High School, St. Louis.
Pomerat, C. M., Assistant in Biology, Clark University.
Rundles, Ralph W., DePauw University.
Scatterty, Louise E., Instructor, H. Sophie Newcomb College.
Sims, D. Lawrence, Student, DePauw University.
Smith, Vera I., Teaching Fellowship, Brown University.
Sullivan, John L., Jr., Johns Hopkins University.
Wagoner, Kenneth S., DePauw University.
Watkeys, Jean De G., Student, University of Rochester.
Wirtz, Sister St. Mark, Instructor in Biology, College of St. Catherine.

PHYSIOLOGY

Bridges, John C., Instructor, Morehouse College.
Butler, Thomas C., Student, Vanderbilt Medical School.
Chao, Iping, the University of Chicago.

Coplan, Helen M., Assistant in Biology, Goucher College.
Craig, Francis N., Rutgers University.
Hess, Margaret, University of Virginia.
Heyl, James T., Hamilton College.
King, Florice A., Graduate Assistant in Physiology, Wellesley College.
McIntire, Josephine M., Assistant, Mount Holyoke College.
Manery, Jeanne F., Student, University of Toronto.
Pappenheimer, Anne, Radcliffe College.
Riedman, Sarah R., Instructor, Department of Physiology, Brooklyn College.
Roeder, Kenneth D., Instructor in Physiology, Tufts College.
Schott, Margaret, Assistant, Mount Holyoke College.
Specht, Heinz, Graduate Student, Johns Hopkins University.
Toothill, Martha C., Instructor in General Biology, Adelphi College.
tum-Suden, Caroline, Research Fellow, Boston University.
Warbritton, Virgene, Research Assistant, University of Missouri.

PROTOZOÖLOGY

Beltran, Enrique, Professor of Zoölogy, University of Mexico.
Bingham, N. Eldred, Instructor, Montclair State Teachers College.
Brown, Rebecca, Goucher College.
Duncan, Paul M., University of Pennsylvania.
Earl, Ruth R., Graduate Student, New York University.
Fuchs, William B., American University.
Kelly, Florene Cora, Instructor, Simmons College.
Lawlor, Sister Anna Catherine, Instructor in Biology, College of Saint Elizabeth.
Levin, Anna C., Columbia University.
Ling, Shao-wen, Graduate Student, The Rockefeller Foundation.
McDonald, Clara M., Nassau Hospital, Minneola.
Manter, John T., Student Assistant, Columbia University.
Metzner, Jerome, College of the City of New York.
Rowland, Claude R., Columbia University.
Smith, Thorn, Jr., Student, Columbia University.
Willis, Doris M., American University.

INVERTEBRATE ZOÖLOGY

Aldinger, Lenore, University of Wisconsin.
Anthony, Genevieve, Graduate Student, University of Pennsylvania.
Axford, Dorothy, Graduate Assistant, New Jersey College for Women.
Bates, M. Noble, Graduate Assistant in Zoölogy, Hamilton College.
Belding, Harwood, Laboratory Assistant, Connecticut State Agricultural College.
Berkenfeld, Charlotte G., Teacher in Elementary Grades, Brooklyn, New York.
Buchheit, J. Robert, Graduate Assistant in Zoölogy, University of Illinois.
Couch, Mary L., Elmira College.
Crooks, Kenneth B. M., Instructor in Biology, Hampton Institute.
Diack, Marion, Student, Oberlin College.
Dibble, Uel E., Graduate Student, Yale University.
Elliott, Alfred M., Teaching Fellow, New York University.
Foltz, Ruth G., Student, Oberlin College.
Goffin, Catherine E., Student, Brown University.
Gray, Beatrice, Student, State University of Iowa.
Grierson, Margaret C., Graduate Student, Mount Holyoke College.
Haffner, Wayne, Wabash College.
Hamilton, Mary A., Student, Elmira College.
Havey, Clinton B., Student, Acadia University.

Henderson, Ruth E., Goucher College.
Hoover, W. Kenneth, Laboratory Assistant, American University.
Huff, George C., State University of Iowa.
Ives, Philip T., Amherst College.
Jacques, Richard, Ohio Wesleyan University.
Jonas, Marion, New Jersey College for Women.
Kleinholz, Lewis H. K., Instructor, Colby College.
Kohn, Henry I., Graduate Student, Yale University.
Larrabee, Martin G., Harvard University.
Ling, Shao-wen, Graduate Student, Cornell University.
Lippy, Grace E., Assistant Professor of Biology, Hood College.
Livengood, Wayne F., Wabash College.
Logan, Amy D., Wilson College.
Lumer, Hyman, Graduate Assistant, Western Reserve University.
Manuel, Beth, Dalhousie University.
Meyer, Adelphia M., Student, Peabody College.
Olsen, Marlow W., Poultry Biologist, United States Department of Agriculture.
Owen, Cora R., Student, Vassar College.
Penn, Amos B. K. C., Graduate Student, Johns Hopkins University.
Pliske, Edward C., Student, Oberlin College.
Rankin, John S., Jr., Student, Wesleyan University.
Reed, Sheldon C., Dartmouth College.
Rees, Olive L., Assistant in Botany, University of Wisconsin.
Sandnes, Gunnar C., College of the City of New York.
Schloemer, Clarence L., Student, Beloit College.
Schoenborn, Henry W., Student, DePauw University.
Setty, Laurel R., Instructor in Biology, Park College.
Shoemaker, Hurst H., Instructor, Earlham College.
Stearns, Mary L., Student, Smith College.
Sures, Pearl M., University of Minnesota.
Tobias, Belle C., Instructor in Biology, Bennett College.
Tukey, Gertrude R., Student, Smith College.
Turner, Robert S., Student, Dartmouth College.
Warren, Marshall R., Student, DePauw University.
Wells, Josephine S., Barnard College.
Zinn, Donald J., Student, Harvard University.

3. TABULAR VIEW OF ATTENDANCE

	1928	1929	1930	1931	1932
Investigators—Total	323	329	337	362	314
Independent	217	234	217	236	212
Under Instruction	81	71	87	83	73
Research Assistants	25	24	33	43	29
Students—Total	133	125	136	125	132
Zoölogy	57	53	56	55	55
Protozoölogy	16	15	14	17	16
Embryology	29	28	27	29	29
Physiology	15	17	23	17	18
Botany	16	12	16	7	14
Total Attendance	456	454	473	487	446
Less Persons registered as both Students and Investigators	2	10	14	20	14
	454	444	459	467	432

INSTITUTIONS REPRESENTED—Total	111	123	126	137	141
By Investigators	80	96	95	102	94
By Students	66	64	71	68	76
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	–	–	–	–
By Students	1	1	4	4	1
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	13	30	7	8	8
By Students	8	3	2	1	1

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1932

Acadia University
Amherst College
Barnard College
Beloit University
Bowdoin College
Brown University
Bryn Mawr College
California Institute of Technology
Carnegie Institution of Washington
Chinese Educational Mission
College of St. Catherine
College of St. Elizabeth
Columbia University
Cornell University
Cornell University Medical College
Dalhousie University
Dartmouth College
DePauw University
Duke University
Elmira College
Fisk University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Eli Lilly & Co.
Long Island University
Memorial Hospital of New York City
Morehouse College
Mount Holyoke College
National Research Council
New York State Department of Health
New York University
New York University Medical School
Oberlin College
Pennsylvania College for Women
Princeton University
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Seton Hill College
Smith College
Sophie Newcomb College
Syracuse University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Iowa
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Vermont
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Washington University
Wellesley College

Wesleyan University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

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5. EVENING LECTURES, 1932

Friday, July 1
Dr. Paul S. Galtsoff .... “The Coral Reefs of the Hawaiian Islands.”

Friday, July 8
Dr. R. W. Gerard .... “The Speed of Life.”

Friday, July 15
Dr. Rudolf Mond .... “Regulation of Ions in the Body Tissues.”

Friday, July 22
Dr. W. E. Garrey .... “Some Aspects of the Physiology of the Heart of Limulus.”

Monday, July 25
Dr. Leif Stoermer .... “Were the Trilobites Related to Limulus?”

Friday, July 29
Dr. C. C. Speidel .... “The Growth and Repair of Living Nerves.”

Friday, August 5
Dr. R. Chambers .... “The Vital Coloration of Protoplasm.”

Friday, August 12
Dr. Alexander Forbes .... “Surveying in Northern Labrador.”

Friday, August 19
The Reynold A. Spaeth Memorial Lecture, delivered by Dr. R. Goldschmidt .... “Genetics and Development.”

Friday, August 26
Dr. H. H. Gran .... “Problems in the Study of the Phytoplankton of the Sea.”

SPECIAL LECTURES AND MOTION PICTURES

Wednesday, July 27
Dr. Paul Weiss (Motion Pictures) .... “The Function of Supernumerary Limbs as Illustrating the Resonance Principle of Nervous Activity.”

Thursday, August 18
Dr. A. R. Moore .... “The Results of Prevention of Membrane Formation in Echinoderm Eggs.”

Wednesday, August 24	
Dr. W. Schopper	"Microcinematographs of Tissue Cultures of Organs from the Guinea Pig."
Friday, August 26	
Dr. C. C. Speidel (Motion Pictures)	"The Growth and Repair of Living Nerves."

6. SHORTER SCIENTIFIC PAPERS, 1932

Tuesday, July 5	
Dr. Ethel B. Harvey	"Splitting of the Eggs of Four Neapolitan Sea Urchins by Centrifugal Force and the Development of the Halves and Quarters."
Dr. Henry J. Fry and Mr. Mark S. Parks	"The Relation between Viscosity Changes and Mitotic Changes in Cleaving Eggs."
Dr. L. V. Heilbrunn	"The Action of Ultra Violet Rays on the Protoplasm of Amœba."
Tuesday, July 12	
Dr. Kenneth S. Cole	"Electric Phase Angle of Tissues."
Dr. Margaret Sumwalt, Dr. W. R. Amberson and Miss Eva Michaelis	"The Part Played by Diffusion Potentials in the Origin of Concentration Potential Differences across Frog Skin."
Dr. E. N. Harvey and Dr. D. A. Marsland	"The Tension at the Surface of Amœba dubia."
Dr. E. N. Harvey	"The Beams Air Turbine for Biological Centrifuging."
Tuesday, July 19	
Dr. A. B. Dawson	"The Relative Degrees of Differentiation of the Mature Erythrocytes of Vertebrates."
Dr. Paul Weiss	"The Factor which Determines the Orientation of the Growing Nerve Fiber."
Dr. G. H. Parker	"Neurohumoralism."
Dr. R. W. Gerard	"Observations on the Velocity of the Nerve Impulse."
Tuesday, July 26	
Dr. M. M. Brooks	"Antagonism of Methylene Blue for CN and CO."
Dr. S. C. Brooks	"Partition Coefficients and Diffusion of Solutes in Heterogeneous Systems."

Dr. A. P. Mathews	"Nature of the Action of Enzymes."
Dr. Laurence Irving and Mr. A. L. Chute	"The Participation of Bone in the Neutralization of Ingested Acid."
Tuesday, August 2	
Dr. W. R. Taylor	"Phytoplankton of Isle Royale, Lake Superior."
Dr. Conway Zirkle	"Cytological Fixation with the Lower Fatty Acids, their Salts, etc."
Dr. G. W. Prescott	"Copper Sulphate as an Algacide in Lakes and Public Water Supplies."
Dr. Albert Saeger	"Manganese and the Growth of Lemnaceæ."
Tuesday, August 9	
Mr. D. P. Costello	"Surface Precipitation Reaction in Marine Eggs."
Dr. P. S. Henshaw	"Changes in Sensitivity of Drosophila Eggs during Early Development to Hard and Soft X-rays, Gamma Rays and Alpha Particles."
Dr. R. M. Buchsbaum	"Size of Explant and Volume of Medium in Tissue Cultures."
Dr. B. H. Willier	"Germ Cells in Relation to the Origin and Differentiation of the Sex Gland of the Chick as Studied by Chorioallantoic Grafts."
Tuesday, August 16	
Dr. Daniel Raffel	"Gene Mutations in Paramecium aurelia."
Dr. C. B. Bridges	"Chromosome Maps of Drosophila."
Dr. A. H. Sturtevant	"A New Unstable Translocation in Drosophila."
Tuesday, August 23	
Dr. T. L. Jahn	"The Effects of Temperature and of Certain Organic Acid Radicals upon Euglena gracilis."
Mr. T. T. Chen	"Nuclear Structure and Mitosis in Zelleriella (Opalinidæ)."
Miss Sabra J. Hook	"Some Observations on Spirostomum ambiguum."
Dr. W. F. Hahnert	"Intensity Duration Relations in the Response of Certain Protozoa to the Electric Current."
Tuesday, August 30	
Dr. A. W. Pollister	"The Development of Leucopoietic Tissue in Amblystoma punctatum."
Dr. W. H. F. Addison and Dr. Doris A. Fraser	"Pigmentation in the Hypophysis and Parathyroids of the Gray Rat."

Dr. George F. Laidlaw	"The Dopa Reaction and the Problem of Pigment Formation in Mammalian Skin."

Friday, September 2

Mr. C. M. Goss, Mr. Bruce Hogg and Dr. Kenneth S. Cole	"Tissue Culture Action Potentials."
Dr. Ethel B. Harvey	"Effects of Centrifugal Force on Fertilized Arbacia Eggs, as Observed with the Microscope Centrifuge."
Dr. P. S. Henshaw	"The Comparative Radiosensitivity of Marine Invertebrate Eggs."
Dr. Margaret Sumwalt	"Anomalous Potential Differences across Frog Skin."
Dr. Walter S. Root	"The Carbon Dioxide Dissociation Curve of Frog's Skeletal Muscle."
Mr. S. A. Corson	"The Effect of Acid and Alkali on the Plasmogel of Amœba proteus."
Dr. F. J. Brinley	"The Action of Salts on Fundulus Embryos."
Dr. Oscar W. Richards	"The Estimation of the Growth of Yeast Populations with a Photoelectric Cell."
Dr. George A. Baitsell	"A Simplified Technique for the Cultivation of Tissues in Vitro."
Dr. T. M. Sonneborn	"Some Genetic Consequences of Self-Fertilization and Cross-Fertilization in Paramecium aurelia."
Dr. E. R. Clark, Mrs. E. L. Clark and Dr. E. A. Swenson	"Motion Pictures Showing the Contraction of Arterioles in the Rabbit's Ear."
Dr. C. C. Speidel	"Moving Pictures of the 'Fast Motion' Type of Various Cells in Living Frog Tadpoles."
Mr. L. V. Beck	"The Effects of Penetrating and Non-Penetrating Acids and Bases on the Oxidation-Reduction Potential of Asterias Ova and of Asterias Sperm."
Dr. G. H. A. Clowes, Miss Anna K. Keltch and Miss Ilene Harryman	"On Inhibition of Maturation of Starfish Eggs by Acids and Acid Producing Agents and the Reversal of this Process by Alkalies."

Miss Anna K. Keltch, Miss Lucille Wade, and Dr. G. H. A. Clowes	"On the Contrasting Sensitivity of Eggs and Sperm to Various Chemical Agents."
Miss Ilene Harryman, Miss Lucille Wade, Miss Anna K. Keltch, and Dr. G. H. A. Clowes	"On the Action of Soaps of the Oleate and Ricinoleate Series on Arbacia Sperm."
Dr. R. Chambers	"On the Formation of the Segmentation Furrow in the Sea Urchin Egg."
Dr. C. G. Pandit	"pH of the Arbacia Egg." (Presented by Dr. R. Chambers.)
Dr. Dorothy R. Stewart and Dr. M. H. Jacobs	"The Influence of Temperature on the Permeability of the Arbacia Egg to Ethylene Glycol."
Dr. Dorothy R. Stewart and Dr. M. H. Jacobs	"The Permeability of the Egg of Asterias to Water."
Mr. Otto Meier, Jr.	"The Use and Cost of Electrical Energy in Relation to Investigators in Attendance at the Marine Biological Laboratory."

7. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Coolidge, Mr. C. A., Ames Building, Boston, Mass.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.

McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
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Morgan, Mrs. T. H., Pasadena, Calif.
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Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
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Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
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Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
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McGee, Dr. Anita Newcomb, Box 363, Southern Pines, N. C.
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Malone, Prof. E. F., University of Cincinnati, Cincinnati, O.
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Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.
Metcalf, Prof. M. M., 94 Nehoiden Road, Waban, Mass.
Metz, Prof. Charles W., Johns Hopkins University, Baltimore, Md.
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Neal, Prof. H. V., Tufts College, Tufts College, Mass.
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Noble, Dr. Gladwyn K., American Museum of Natural History, New York City, N. Y.

Nonidez, Dr. José F., Cornell University Medical College, New York City, N. Y.
Okkelberg, Dr. Peter, University of Michigan, Ann Arbor, Mich.
Osburn, Prof. R. C., Ohio State University, Columbus, O.
Osterhout, Prof. W. J. V., Rockefeller Institute, New York City, N. Y.
Packard, Dr. Charles, Columbia University, Institute of Cancer Research, 1145 Amsterdam Ave., New York City, N. Y.
Page, Dr. Irvine H., Rockefeller Institute, New York City, N. Y.
Papanicolaou, Dr. George N., Cornell University Medical College, New York City, N. Y.
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Paton, Prof. Stewart, Princeton University, Princeton, N. J.
Patten, Dr. Bradley M., Western Reserve University, Cleveland, O.
Patten, Prof. William, Dartmouth College, Hanover, N. H.
Payne, Prof. F., University of Indiana, Bloomington, Ind.
Pearl, Prof. Raymond, Institute for Biological Research, 1901 East Madison Street, Baltimore, Md.
Pearse, Prof. A. S., Duke University, Durham, N. C.
Peebles, Prof. Florence, California Christian College, Los Angeles, Calif.
Phillips, Dr. E. F., Cornell University, Ithaca, N. Y.
Pike, Prof. Frank H., 437 West 59th Street, New York City, N. Y.
Pinney, Dr. Mary E., Milwaukee-Downer College, Milwaukee, Wis.
Plough, Prof. Harold H., Amherst College, Amherst, Mass.
Pollister, Dr. A. W., Columbia University, New York City, N. Y.
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Raffel, Dr. Daniel, Zoölogical Laboratory, Johns Hopkins University, Baltimore, Md.
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de Renyi, Dr. George S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.

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Riggs, Mr. Lawrason, Jr., 25 Broad Street, New York City, N. Y.

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Romer, Dr. Alfred S., The University of Chicago, Chicago, Ill.

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Sayles, Dr. Leonard P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, N. Y.

Schrader, Dr. Franz, Department of Zoölogy, Columbia University, New York City, N. Y.

Schrader, Dr. Sally Hughes, Department of Zoölogy, Columbia University, New York City, N. Y.

Schramm, Prof. J. R., University of Pennsylvania, Philadelphia, Pa.

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Scott, Prof. John W., University of Wyoming, Laramie, Wyo.

Scott, Prof. William B., 7 Cleveland Lane, Princeton, N. J.

Severinghaus, Dr. Aura E., Department of Anatomy, College of Physicians and Surgeons, 630 W. 168th Street, New York City, N. Y.

Shull, Prof. A. Franklin, University of Michigan, Ann Arbor, Mich.

Shumway, Dr. Waldo, University of Illinois, Urbana, Ill.

Sivickis, Dr. P. B., Pasto deze 130, Kaunas, Lithuania.

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Snyder, Prof. Charles D., Johns Hopkins University Medical School, Baltimore, Md.

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Stark, Dr. Mary B., New York Homeopathic Medical College and Flower Hospital, New York City, N. Y.

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Stockard, Prof. C. R., Cornell University Medical College, New York City, N. Y.

Stokey, Dr. Alma G., Mount Holyoke College, South Hadley, Mass.

Strong, Prof. O. S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.

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Waite, Prof. F. C., Western Reserve University Medical School, Cleveland, O.

Wallace, Dr. Louise B., 523 Cowper Street, Palo Alto, Calif.

Ward, Prof. Henry B., University of Illinois, Urbana, Ill.

Warren, Dr. Herbert S., Department of Biology, Temple University, Philadelphia, Pa.

Warren, Prof. Howard C., Princeton University, Princeton, N. J.

Wenrich, Dr. D. H., University of Pennsylvania, Philadelphia, Pa.

Whedon, Dr. A. D., North Dakota Agricultural College, Fargo, N. D.

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Wilson, Dr. J. W., Brown University, Providence, R. I.
Witschi, Prof. Emil, University of Iowa, Iowa City, Ia.
Woglom, Prof. William H., Columbia University, New York City, N. Y.
Woodruff, Prof. L. L., Yale University, New Haven, Conn.
Woodward, Dr. Alvalyn E., Zoölogy Department, University of Michigan, Ann Arbor, Mich.
Young, Dr. B. P., Cornell University, Ithaca, N. Y.
Young, Dr. D. B., University of Maine, Orono, Me.
Zeleny, Dr. Charles, University of Illinois, Urbana, Ill.

ISOLATION OF COLPIDIUM STRIATUM STOKES IN BACTERIA-FREE CULTURES AND THE RELATION OF GROWTH TO pH OF THE MEDIUM

ALFRED M. ELLIOTT

Biological Laboratory, University College, New York University

Introduction

Until rather recently, the many attempts to grow ciliates in bacteria-free cultures have met with little success. One of the first unquestioned cases is that of *Glaucoma piriformis,* which was isolated by Lwoff in 1922 and has been maintained in bacteria-free cultures for the past ten years (Lwoff, 1932). Peters (1921) had previously reported the establishment of bacteria-free cultures of *Colpidium colpoda,* but his results were criticised by Oehler (1924), who pointed out that the tests for bacteriological purity of the cultures were inadequate. Peters himself later reported (Gatenby and Cowdry, 1928, pp. 412–413) the recovery of bacteria from his cultures of *Colpidium.* Oehler, however, isolated strains of *Colpoda steinii* in 1916 and *C. cucullus* in 1923 (Oehler, 1924), and was able to maintain them on spinach broth. Milkovitch (1929) isolated another strain of *Glaucoma piriformis,* using a medium somewhat more complicated than that of Lwoff. In 1930 Glaser and Coria reported the establishment of bacteria-free strains of *Trichoda pura* and *Paramecium caudatum,* and more recently (Glaser, 1932) *Paramecium multimicronucleatum* has been added to the list. In addition, Hetherington (1932) has reported the establishment of bacteria-free cultures of *Colpidium campylum, Glaucoma scintillans* and *Loxocephalus sp.*

In the present investigation the writer has isolated *Colpidium striatum* in bacteria-free cultures and, as a necessary preliminary to further investigations, has attempted to devise satisfactory culture methods and to determine the relation of growth to pH of the medium. The writer wishes to express his appreciation to Professor R. P. Hall for suggestions during the course of the investigation.

Material and Methods

The bacteria-free strain of *Colpidium striatum* used in this investigation is a pure line derived from a single organism which was isolated from a hay infusion culture. By employing sterile micropipettes, depression slides, Petri dishes and a 0.25 per cent tryptone medium a single organism was washed free of bacteria in the manner described by Parpart (1928) for *Paramecium.* It was found that when the bacteria were entirely eliminated, the *Colpidium* gradually decreased in size and then died after about two days. During the washing process, however, when a few bacteria were still present, the organism usually divided several times. These results finally led to the following procedure. A single ciliate was transferred to a tube of dextrose broth along with a few bacteria; abundant growth of the ciliates occurred. From this tube others were inoculated by the loop method, each loop containing approximately 10–20 ciliates together with a few bacteria. Growth of the ciliates was maintained through several series of such transfers, and by the plating method it was noted that the bacterial count was gradually decreasing. It was obvious at this point that the bacteria were not the sole source of food for the ciliates, since the concentration of protozoa was many times as great as that of the bacteria. This period of subculturing might be considered a sort of " acclimatization period " during which the number of bacteria is greatly reduced and the ciliates become more and more dependent upon nutritive substances in solution. After a number of series of such subinoculations, agar plates inoculated (1.0 cc.) from a number of culture tubes failed to show any bacterial colonies (duplicate series of plates were incubated at 37° C. and at room temperature for 10 days). In previous tests the presence of bacteria had been distinctly evident after 48 hours at 37° and after 4 days at room temperature. In the case of plates which showed no bacterial colonies, the colonies of ciliates were examined by the usual bacteriological staining methods as a confirmatory test. These bacteria-free ciliate colonies were then transplanted to liquid media, in which growth has since been maintained. Subsequent tests for bacteria, repeated at intervals over a period of several months, have been consistently negative.

Although a comparatively slow procedure, the method of isolation just described may have certain advantages. Most investigators have attempted to transfer ciliates directly from bacterized cultures to sterile media, and have failed to obtain growth in many cases; similarly, the writer's attempts to use this method were unsuccessful with *Colpidium* and several other ciliates. The more gradual elimination of bacteria by the dilution method was effective in the case of *Colpidium* and may prove to be so for other ciliates which cannot survive a sudden transfer from heavily bacterized to bacteria-free media.

In attempts to determine types of media satisfactory for growth of *Colpidium striatum* various kinds of Difco peptones and bacteriological media were tried. Difco dextrose broth gave excellent results, with maximum populations of approximately 200,000 per cc. Inoculation of Durham fermentation tubes containing phenol-red dextrose broth was followed by acid fermentation in 24 hours, indicating that *Colpidium* utilizes dextrose readily. Difco nutrient gelatin and litmus milk were fairly satisfactory. In the gelatin tubes stratiform liquefaction was evident after 24 hours (room temperature), and the depth of the liquefied zone increased steadily throughout the period of observation (5 days). In litmus milk the casein was coagulated and the indicator reduced after about 48 hours, and complete peptonization had occurred in about two weeks. It is obvious, therefore, that *Colpidium striatum* produces a gelatinase and an enzyme capable of hydrolyzing casein. A similar gelatinase has previously been reported by Lwoff (1932) in *Glaucoma piriformis*. Of the various peptones tested, Difco tryptone proved to be very satisfactory, and has therefore been used in some of the experiments described below.

For experimental purposes the ciliates were grown in 16 × 150 mm. Pyrex tubes plugged with cotton. With the exception of Series I, experimental cultures were incubated at a temperature of 28° C. in a water bath, the cultures being inclined on a wire rack at an angle of 45 degrees, 18 inches below a battery of six 100-watt light bulbs. In tubing the media, 9.6 cc. of the appropriate medium at the desired pH were measured directly into the culture tube by means of a Schellbach burette graduated to 0.1 cc.; after sterilization in the autoclave, the amount of medium in each tube was approximately 9.0 cc. The pH of the medium was adjusted by means of a LaMotte roulette comparator, readings being subject to an error of ± 0.1.

In determining amounts of growth, initial and final counts were made with a Sedgwick-Rafter counting chamber and a Whipple micrometer.

GROWTH IN RELATION TO pH OF THE MEDIUM

Series I

In this preliminary series to determine the optimum pH and growth range for *Colpidium,* the following medium was used:

KNO_3	0.5	gram
KH_2PO_4	0.5	"
$MgSO_4$	0.25	"
NaCl	0.10	"
$NaC_2H_3O_2$	1.5	"
Difco tryptone	3.0	"
Distilled water	1.0	liter

The addition of sodium acetate to the medium was expected to increase the growth rate of *Colpidium,* since this substance has been shown (Mainx, 1928; Jahn, 1932; Lwoff, 1932; Loefer, MSS) to have such an effect on certain flagellates; later findings, however, have indicated that the reverse is true for *Colpidium*. The pH of the different sets of tubes after inoculation was determined (Table I), and the series was grown at room temperature for 96 hours; half of the tubes in each set being counted at 48 hours and the remainder at 96 hours. The initial count was 234 organisms per cubic centimeter; the 48-hour and 96-hour counts are indicated in Fig. 1. Very little, if any, growth occurred in the tubes having an initial pH of 5.0, 5.5 and 9.1; and there was no growth below pH 5.0.

In determining the pH of the various sets of tubes after 48 and 96 hours of incubation, respectively, it was noted that distinct changes had been produced in the majority of instances (Table I). The tubes with an initial pH of 7.5 and below showed a decrease in hydrogen ion concentration; those of 8.0 and higher showed an increase.

The counts indicated that maximum growth had occurred in the tubes with an initial pH of 6.6; however, the pH of these tubes had risen to 7.3 at 48 hours and to 8.2 at 96 hours. It was apparent that the medium was not buffered sufficiently to maintain a reasonably constant pH during the period of incubation, and in later series the composition of the medium was changed in attempts to meet this difficulty.

Series II

The medium used in this series was made up with increased amounts of buffer substances in the hope of preventing some of the pH changes noted in Series I:

KNO_3	0.5	gram
KH_2PO_4	1.0	"
$MgSO_4$	0.25	"
NaCl	0.10	"
Difco tryptone	8.0	"
Distilled water	1.0	liter

With an initial count of 1630 per cc., the pH of the various sets of tubes (Table II) ranged from 3.5 to 9.7. The series was incubated for 96 hours in a water bath at a constant temperature of 28.0° C. Although changes in pH (Table II) were noted in the tubes of this series also, they were not quite so extensive as in Series I.

The growth range, in this series, extended from 4.2 to 8.6 (initial pH), although very little growth occurred in the tubes at the two ends of the range. A more interesting feature of this series is the fact that, at 48 hours, maximum growth (Fig. 2) had occurred in the tubes with an initial pH of 5.5; those at 6.9 ranked second; while the tubes at 6.2 and 6.4 showed distinctly less growth. At 96 hours the maximum growth had shifted to the tubes with an initial pH of 6.4, while those at 5.5

ranked second, with a distinctly lower count at 6.2. These unexpected results were complicated by pH changes in the medium during growth of the ciliates, but they suggest that two different pH zones are favorable to growth of *Colpidium,* a condition which was not apparent in the medium (containing sodium acetate) used in Series I.

TABLE I

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
3.5	3.8	3.9	0.4
4.2	4.3	4.3	0.1
4.6	4.7	4.7	0.1
5.0	5.4	5.4	0.4
5.5	5.5	5.5	0.0
6.2	6.9	7.8	1.6
6.6	7.3	8.2	1.6
7.2	7.7		
7.5	7.8	8.0	0.5
8.0	7.9	7.9	−0.1
8.4	7.9	7.9	−0.6
8.6	7.9		
9.1	8.5	8.5	−0.6

TABLE II

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
3.5	3.5	3.5	0.0
4.2	4.2	4.2	0.0
4.6	4.6	4.6	0.0
5.0	5.0	5.2	0.2
5.5	6.5	6.6	1.1
6.2	7.1	7.2	1.3
6.4	7.3	7.5	1.2
6.9	7.5	7.7	0.8
7.5	7.7	7.8	0.3
8.2	7.9	7.9	−0.3
8.4	8.2	8.2	−0.2
8.6	8.5	8.4	−0.2
9.7	9.7	9.7	0.0

Series III

In view of the differences between Series I and II it was decided to compare growth with and without sodium acetate, and also to add more tryptone to the medium in the hope of decreasing the pH changes. The medium was the same as that in Series II, except that 10 grams of tryptone were used instead of 8. To half of the medium, sodium acetate was added in a concentration of 0.2 per cent, and the pH of different portions of each was adjusted to the desired points. The initial pH of each set of tubes (Table III) was determined after inoculation. The initial count was 300 per cc. Counts were made (Fig. 3) after incubation for 96 hours in the water bath.

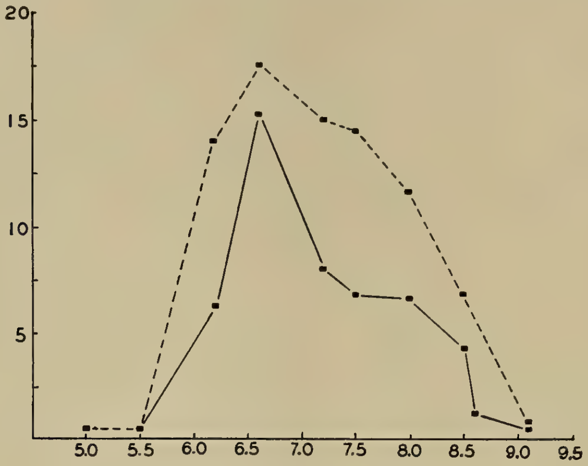

FIG. 1. Concentration of ciliates in thousands per cubic centimeter is plotted against initial pH. The solid line indicates growth at 48 hours; the broken line, growth at 96 hours.

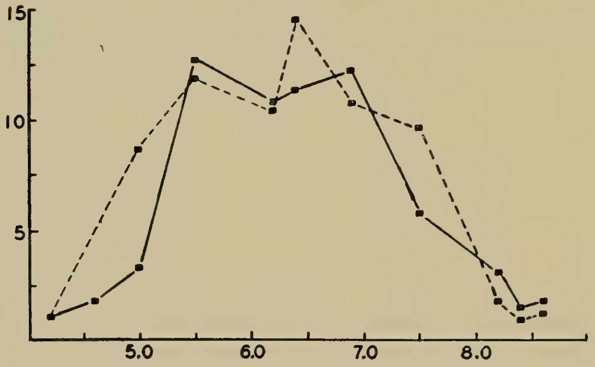

FIG. 2. Series II. Final concentration in thousands per cubic centimeter plotted against initial pH. The solid line indicates growth at 48 hours; the broken line, growth at 96 hours.

The final counts showed clearly that sodium acetate exerts a distinct effect on *Colpidium striatum* but that, contrary to expectations, the

TABLE III

With sodium acetate			Without sodium acetate		
Initial pH	pH at 96 hours	Change in pH	Initial pH	pH at 96 hours	Change in pH
3.5	3.5	0.0	3.5	3.5	0.0
4.0	4.0	0.0	3.8	3.8	0.0
4.5	4.5	0.0	4.5	4.5	0.0
5.2	5.2	0.0	5.0	5.0	0.0
5.5	5.5	0.0	5.5	5.5	0.0
6.2	6.2	0.0	5.7	5.8	0.1
6.4	6.4	0.0	6.5	7.0	0.5
7.0	7.5	0.5	7.0	7.5	0.5
7.8	7.9	0.1	7.6	7.6	0.0
8.2	8.2	0.0	8.0	8.0	0.0
8.6	8.6	0.0	8.2	8.2	0.0
8.8	8.8	0.0	8.6	8.7	0.1
9.5	9.5	0.0	9.5	9.5	0.0

growth rate is decreased rather than increased as in Euglenida and Phytomonadida. In addition, the pH range which permits growth is narrower than in the medium without sodium acetate: 6.2 to 7.8 in the acetate medium, and 5.0 to 8.0 in the medium without acetate. While maximum growth occurred in the tubes with an initial pH of 7.0 for each type of medium, the medium without acetate showed a second high point

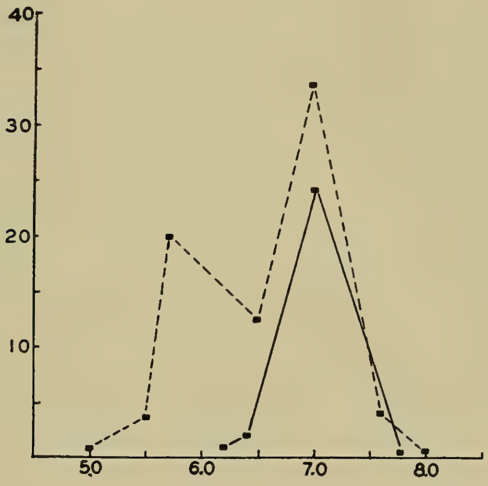

FIG. 3. Series III. Concentration of ciliates in thousands per cubic centimeter is plotted against initial pH. The solid line indicates concentration in the sodium-acetate medium; the broken line, the medium without sodium acetate.

at pH 5.7, a type of growth similar to that noted previously in Series II. As indicated in Table III, the pH changes were relatively slight in this more heavily buffered medium; hence, it seems obvious that there are two " optima " for growth of *Colpidium* in the medium containing no sodium acetate, whereas only a single optimum is noted in the acetate medium (see also Series I).

Series IV

At this point in the investigation it seemed desirable to determine whether the " bimaximal " type of growth could be observed in a different medium. Consequently, Difco dehydrated dextrose broth (5 grams) was substituted for tryptone in the medium used in Series I. The initial count was 900 organisms per cubic centimeter. Preliminary counts were made at 48 hours, and final counts (Fig. 4) at 96 hours of incubation in the water bath.

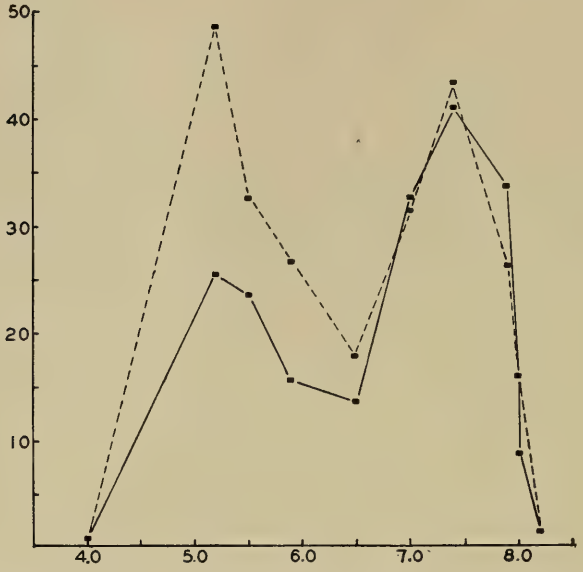

FIG. 4. Series IV. Concentration of organisms in thousands per cubic centimeter is plotted against initial pH. The solid line indicates concentration at 48 hours; the broken line, at 96 hours.

At 48 hours maximum growth had occurred in the tubes with an initial pH of 7.4, with a second distinct high point at 5.2. At the end of 96 hours, growth in the 5.2 tubes had exceeded that at 7.4. In this case the bimaximal type of growth was even more evident than in the preceding series; allowing for observed changes in pH (Table IV), the lower " maximum " in this case lies between pH 5.2 and 5.5, and the upper between 7.4 and 7.6.

Table IV

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
4.0	4.0	4.0	0.0
5.2	5.4	5.5	0.3
5.5	6.5	6.8	1.3
5.9	6.7	7.0	1.1
6.5	7.0	7.2	0.8
7.0	7.2	7.5	0.5
7.4	7.5	7.6	0.2
7.9	7.6	7.7	−0.2
8.0	7.7	7.7	−0.3
8.2	7.8	7.8	−0.4
8.2	7.8	7.8	−0.4

BIMAXIMAL GROWTH IN RELATION TO FINAL pH

In relation to initial pH of the medium, the growth "maxima" (as indicated by final counts) were as follows: Series II, 5.5 and 6.4; Series III, 5.7 and 7.0; Series IV, 5.2 and 7.4. On comparing the final counts with the final pH, the maxima lie as follows (Fig. 5): Series II, 6.6 and 7.5; Series III, 5.8 and 7.5; Series IV, 5.5 and 7.6. In Series II, the initial pH 6.4 had shifted to 7.3 at 48 hours, so it seems obvious that in

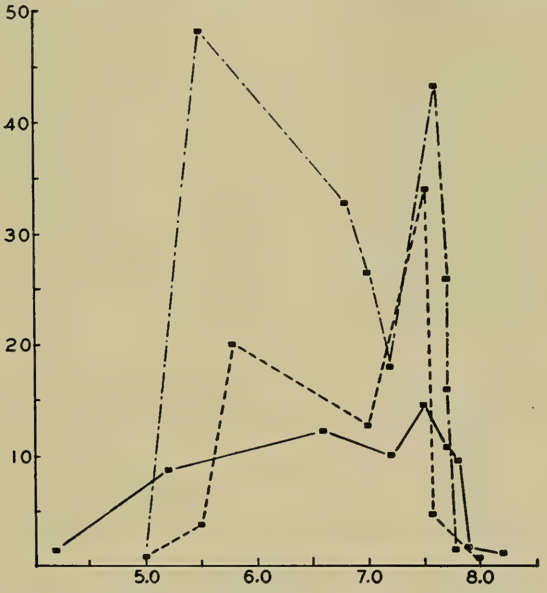

Fig. 5. Concentration of organisms in thousands per cubic centimeter is plotted against final pH in Series II (————), III (— — — — —) and IV (— - — - — - —).

the growth of *Colpidium striatum* in relation to pH, one optimum lies above 7.0 (perhaps near 7.5–7.6), while the other lies in the acid range, the lowest optimum (5.5) being observed in dextrose broth medium.

Division Rate in Relation to Final pH

Conflicting results have been reported concerning the relation of hydrogen-ion concentration to division rate in ciliates. Darby (1929) demonstrated, within the limits of his experiments, that *Paramecium caudatum* and *P. aurelia* show a marked difference in division rate in media of varying pH values. Phelps (1931), however, found that *P. aurelia,* grown on one strain of bacteria, showed no difference in division rate between pH limits of 5.9 and 7.7. He attributed any change in fission rate, within these limits, to a change in the available bacterial food and not to the direct action of pH. However, in comparing the average fission rate per day of *Colpidium striatum,* calculated from final counts using the formula,

$$n = \frac{\log b - \log B}{\log 2},$$

with final pH, the following differences were noted: In Series II, at pH 5.2 and 7.8 the fissions per day were 0.60 and 0.64 respectively; while at the optima, pH 6.6 and 7.5, the rates were 0.72 and 0.80. In Series III, at pH 5.0 and 7.6 the fission rates were 0.36 and 0.94; at the optima, pH 5.8 and 7.5, the rates were 1.52 and 1.71. Likewise in Series IV, at pH 5.5 and 7.7, the fission rates were 1.44 and 1.04 respectively; at the alkaline optimum (pH 7.6), 1.39 fissions per day. These results indicate that, within the range mentioned by Phelps, the division rate of *Colpidium striatum* varies with pH of the medium, as previously maintained by Darby (1929) for *Paramecium.*

Discussion

The literature on the relation of hydrogen-ion concentration to the growth of ciliates is not very extensive, and at the same time somewhat contradictory results have in certain instances been reported for the same species. A survey of the information available indicates that *Colpidium striatum* is tolerant to a somewhat wider range of hydrogen-ion concentration than is the case for a number of other ciliates. On the basis of the results described above, *C. striatum* will grow in media with initial pH of 4.0 to 8.6, with a narrower range in a medium containing sodium acetate. The range is somewhat broader than that (approximately 6.0 to 8.5) described by Pruthi (1926) for *Colpidium sp.,* but agrees fairly well with the findings of Mills (1931) that the rate of food-vacuole formation increases from pH 4.5 to 6.0 and then decreases to zero at pH 8.0. Other ciliates show a more restricted pH range. For example, the range for *Spirostomum ambiguum,* as reported by Saunders

(1924), is pH 6.8 to 7.8, and according to Morea (1927), pH 6.5–8.0. Darby (1929) stated that in *Paramecium caudatum* division occurs between pH 5.3 and 8.2; *P. aurelia*, according to the same author, grows at pH 5.7–7.8, while Morea (1927) stated that this species would live at pH 6.0 to 9.5. On the other hand, *P. multimicronucleatum* is more nearly comparable with *Colpidium striatum*, since it grows at pH 4.8–8.3 (Jones, 1930). An interesting feature in the growth of *Colpidium striatum* is the presence of two pH "optima." Loefer (1932) has noted a similar situation in the growth of *Chilomonas*. Hopkins (1928), in comparing the relation of pH to rate of locomotion in *Amœba proteus*, obtained a double optimum with an intermediate low point at pH 7. Likewise, Mast (1931), in comparing length of life with pH in the same form, observed one optimum at pH 6.6–7.0 and another at 5.0–6.4. In growth of *Colpidium striatum* one optimum appears above pH 7.0 and another below pH 7.0. An explanation of this bimaximal type of growth range in Colpidium is not yet available, but it may possibly involve the activities of different types of enzymes elaborated by the ciliates.

The effect of sodium acetate on *C. striatum* was surprising. Various investigators (Mainx, 1928; Jahn, 1932; Lwoff, 1932; Loefer, MSS) are agreed that this substance greatly accelerates the growth of certain flagellates. Such reports have been confirmed by the writer in the case of *Hæmatococcus pluvialis;* this flagellate was grown in the acetate medium used for *Colpidium* as a check on the results obtained with the ciliate—growth of *Hæmatococcus* was distinctly accelerated by sodium acetate. Collett (1919), however, observed that the acetate radical, among others, was toxic to both *Paramecium* and *Euplotes*. Mast (1931) also found that acetate in N/1000 concentration was more toxic than several other anions ($—SO_4$, $—NO_3$, $—Cl$, $—PO_4$). In the case of *C. striatum*, addition of sodium acetate to the medium brings about a definite depression of the division rate. At the same time, the growth range in relation to pH is distinctly narrowed, and the two pH "optima" (seen in media without acetate) are replaced by a single optimum. The significance of these peculiar effects of acetate on *Colpidium* is still unknown.

SUMMARY

The method used in isolating *Colpidium striatum* in bacteria-free cultures is outlined, and suitable culture media are described. Growth of the ciliates was found to occur within the pH range 4.0 to 8.6. Within this range two growth "maxima" were observed, one above and one below pH 7.0. Addition of sodium acetate to the medium restricted the growth range, depressed the fission rate, and brought about

a replacement of the two growth "maxima" by a single maximum. Division rate was found to vary with the pH of the medium. Acid fermentation of dextrose, liquefaction of gelatin, and peptonization of litmus milk were observed.

LITERATURE CITED

Collett, M. E., 1919. The Toxicity of Acids to Ciliate Infusoria. *Jour. Exper. Zoöl.,* **29**: 443.

Darby, H. H., 1929. The Effect of Hydrogen-ion Concentration on the Sequence of Protozoan Forms. *Arch. f. Protist.,* **65**: 1.

Gatenby, J. B., and E. V. Cowdry, 1928. Bolles Lee's Microtomist's Vade-mecum (Philadelphia, Blakiston).

Glaser, R. W., 1932. Cultures of Certain Protozoa, Bacteria-free. *Jour. Parasit.,* **19**: 173 (Abstract).

Glaser, R. W., and N. A. Coria, 1930. Methods for the Pure Culture of Certain Protozoa. *Jour. Exper. Med.,* **51**: 787.

Hetherington, A., 1932. The Constant Culture of Stentor cœruleus. *Arch. f. Protist.,* **76**: 118.

Hopkins, D. L., 1928. The Effects of Certain Physical and Chemical Factors on Locomotion and Other Life Processes in Amœba proteus. *Jour. Morph.,* **45**: 97.

Jahn, T. L., 1932. The Effect of Certain Organic Acid Radicals on Growth of Euglena gracilis. *Anat. Rec.,* **54** (Suppl.) : 42 (Abstract).

Jones, E. P., 1930. Paramecium Infusion Histories. I. Hydrogen-ion Changes in Hay and Hay-flour Infusions. *Biol. Bull.,* **59**: 275.

Loefer, J. B., 1932. Relation of Hydrogen-ion Concentration to Growth of Chilomonas. *Anat. Rec.,* **54** (Suppl.) : 102 (Abstract).

Lwoff, A., 1932. Recherches biochemiques sur la nutrition des Protozoaires. Le pouvoir synthèse. Monographies de l'Institute Pasteur (Paris, Masson et Cie), 158 pp.

Mainx, F., 1928. Beiträge zur Morphologie und Physiologie der Eugleninen. I. Teil. Morphologische Beobachtungen, Methoden und Erfolge der Reinkultur. II. Teil. Untersuchungen über die Ernährungs- und Reizphysiologie. *Arch. f. Protist.,* **60**: 305.

Mast, S. O., 1931. Effects of Salts, Hydrogen-ion Concentration, and Pure Water on Length of Life in Amœba proteus. *Physiol. Zoöl.,* **4**: 58.

Milkovitch, G., 1929. Action du sérum humain sur un infusoire, Glaucoma piriformis. *Compt Rend. Soc. Biol.,* **100**: 417.

Mills, S. M., 1931. The Effect of the Hydrogen-ion Concentration on Protozoa, as Demonstrated by the Rate of Food Vacuole Formation in Colpidium. *Brit. Jour. Exp. Biol.,* **8**: 17.

Morea, L., 1927. Influence de la concentration en ions H sur la culture de quelques Infusoires. *Compt. Rend. Soc. Biol.,* **97**: 49.

Oehler, R., 1924. Weitere Mitteilungen über gereinigte Amöeben- und Ciliatenzucht. *Arch. f. Protist.,* **49**: 112.

Parpart, A. K., 1928. The Bacteriological Sterilization of Paramecium. *Biol. Bull.,* **55**: 113.

Peters, R. A., 1921. The Substances Needed for the Growth of a Pure Culture of Colpidium colpoda. *Jour. Physiol.,* **55**: 1.

Phelps, A., 1931. Effect of Hydrogen-ion Concentration on the Division Rate of Paramecium aurelia. *Science,* **74**: 395.

Pruthi, H. S., 1926. On the Hydrogen-ion Concentration of Hay Infusions, with Special Reference to its Influence on the Protozoan Sequence. *Brit. Jour. Exper. Biol.,* **4**: 292.

Saunders, J. T., 1924. The Effect of the Hydrogen-ion Concentration on the Behavior, Growth and Occurrence of Spirostomum. *Biol. Rev.,* **1**: 189.

MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

I. DECOMPOSITION OF MARINE PLANT AND ANIMAL RESIDUES BY BACTERIA[1]

SELMAN A. WAKSMAN, CORNELIA L. CAREY,[2] AND HERBERT W. REUSZER

(From the Woods Hole Oceanographic Institution and the New Jersey Agricultural Experiment Station)

The synthesis of organic matter in the sea through the agency of the chlorophyll-bearing plants, ranging from the smallest diatoms to the largest algæ, consists in the assimilation of the carbon as CO_2, of the nitrogen as nitrate, and probably to a lesser extent as ammonia and nitrite, of the phosphorus as phosphate, and of other elements in lower concentrations. Before these elements can be returned to circulation, after the plants as well as the animals which fed partly upon them die, the complex organic substances have to be decomposed through the agency of bacteria. If one is to judge by analogy with the decomposition processes which take place on land, the liberation of the elements in a mineralized form does not represent a simple process, but rather a chain of processes. The rate of liberation of the elements by bacterial action depends primarily upon the chemical composition of the materials undergoing decomposition and upon the organisms active in the decomposition processes.

The following investigations were undertaken for the purpose of determining to what extent the chemical composition of marine residues of plant and animal origin influences the rate of their decomposition by marine bacteria and the rate of liberation of the important elements, especially nitrogen, in an available form. It was further essential to obtain light upon the mechanism of decomposition of some of the chemical constituents of the marine residues by specific members of the bacterial population of the sea. These investigations can be classified, therefore, under three distinct headings, namely: (1) the chemical composition of marine zoöplankton and of certain marine algæ, (2) the decomposition of the plankton and algal material in sea water and in marine

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[2] Department of Botany, Barnard College, Columbia University.

mud by bacteria, (3) the decomposition of certain algal constituents by specific bacteria isolated from the sea.

Materials Used and their Chemical Composition

Two types of organic matter of marine origin were used in this investigation, one representing zoöplankton and one algal material. The zoöplankton was collected on July 6, 1932, in Buzzard's Bay, by means of an ordinary scrim plankton net. As soon as brought to the laboratory, the excess liquid was pressed out through cheesecloth and through a fine silk net, and plankton then dried in a current of warm air. The plankton consisted largely of copepods (*Centropages hamatus* and *C. typicus*), a few isopods, decapods, *Sagitta elegans,* fragments of hydroids, and fish eggs. The liquid pressed out by ordinary hand pressure from the original plankton was examined and found to consist largely of bacteria and some colloidal material.

The air-dry plankton contained 6.7 per cent moisture. This material was subjected to analysis and the results calculated on a dry basis. The total dry plankton was found to consist of 7.88 per cent nitrogen, 28.1 per cent ash, of which 15.1 per cent was sea salt as calculated from the chlorine content. The complete proximate analysis (22) of the plankton is given in Table I.

Table I

Proximate analysis of zoöplankton. Dry, salt-free basis.

Chemical constituent	Percentage
Ether-soluble fraction *	10.1
Alcohol-soluble, non-nitrogenous *	4.8
Cold water-soluble *	3.1
Hot water-soluble *	2.0
Chitin	5.8
Protein	55.7
Cellulose	2.4
Lignin (?)	2.8
Ash, salt-free	13.0
Total accounted for	99.7

* Ash and nitrogen-free basis.

The chitin content was determined on a separate sample, by repeated treatment with hot dilute hydrochloric acid (2 per cent) and hot 12 per cent KOH solution, then washing with alcohol and ether. The nitrogen of the chitin was subtracted from the total nitrogen of the material and the rest calculated as protein, using the factor 6.25. The cellulose content was calculated from the amount of sugar produced on hydrolysis of the plankton residue (after extraction with hot dilute acid) with 80 per cent sulfuric acid in the cold, diluting with 15 volumes of water and heating in order to complete the hydrolysis. The total sugar formed on hydrolysis was corrected for the reducing sugar produced from the chitin and calculated as cellulose, using the factor 0.9. The cold water extract of the plankton was free from reducing sugar, while the hot water and the hot dilute acid extracts were free from glycogen and hemicellulose. Just as in the case of the alcohol extract, the cold and hot water-soluble fractions were corrected for ash and nitrogenous constituents.

The danger of using alcohol for the preservation of the plankton is brought out by the fact that when treated with 95 per cent ethyl alcohol, the total plankton gave 24.7 per cent alcohol-soluble material, of which 23.2 per cent was organic matter. The low figure reported in the table for the alcohol-soluble fraction is due to the fact that in the complete analysis the dry plankton was first extracted with ether, while the total alcohol-soluble portion was corrected for ash and protein, so as to avoid duplicating the same constituent in the final analysis; since a large part of the ether-soluble material and some of the nitrogenous constituents are also soluble in alcohol, the corrections made resulted in reducing the concentration of the remaining alcohol-soluble fraction.

The results of this analysis are quite in accord with similar analyses made by Brandt (2), who employed, however, the methods commonly used in foodstuff analysis, whereby the chemical constituents were reported as crude fat, protein, carbohydrate, crude fiber, and nitrogen-free extractives.

It is important to call attention, in connection with this analysis, to the high content of organic nitrogenous constituents in the zoöplankton as well as of fatty substances and of chitin, and to the low content of carbohydrates. The amount reported as cellulose is so small that one may be justified in questioning whether this represents true cellulose or other complexes, which give, in addition to chitin, reducing substances on hydrolysis with concentrated acid; the presence of a small amount of cellulose may also be due to certain constituents of plant origin. The same is true of the lignin; the concentration of this complex is so small that its origin in the plankton may still be considered as an open question; it may actually represent a certain polyuronide not readily hydrolyzed by 80 per cent sulfuric acid in the cold (14).

As sources of algal material, *Fucus vesiculosus, F. platycarpus,* and *Ulva lactuca* were selected. In the case of the *Fucus,* the material was brought to the laboratory as soon as collected, cleaned thoroughly by hand to remove extraneous matter, washed 2–3 times with tap water, then with distilled water to remove the excess of salt, and dried at about 80–90° C. In the case of the *Ulva,* however, the material was dried directly, without preliminary washing. The results of the analysis of these three algal materials are reported in Table II.

The cold water-soluble fraction of the *Fucus* contained 1 to 2.5 per cent reducing sugar, on the basis of the total dry material. The hot water-soluble fraction, when hydrolyzed with dilute acid, gave 2.8 to 3.5 per cent reducing sugar, pointing to the presence in this fraction of complex carbohydrates. After the hot water extraction, the material was treated with 2 per cent hydrochloric acid, at 100° C., for 5 hours; the extract was removed by filtration, neutralized and analysed for reducing sugar; the results obtained are reported as " hemicelluloses." However, this figure does not represent the total hemicellulose, including both true polysaccharides and polyuronides; when separate samples were analyzed for uronic acid and for total pentosan (furfuraldehyde method), considerably larger figures were obtained than those given under " hemicelluloses." This is due to the fact that the furfuraldehyde formed on treatment of the material with 12 per cent hydrochloric acid may be due to substances consisting not only of pentoses derived from pentosans but also of uronic acid complexes forming polyuronides. The latter change on hydrolysis only incompletely to reducing sugar. The three sets of results for the carbohydrate groups, namely the reducing sugar formed on hydrolysis with dilute acid, the pentosan, and the uronic acid anhydride, overlap and render it, therefore, impossible to make a final addi-

TABLE II

Chemical composition of three marine algæ. Dry, salt-free basis.

Organism	*Fucus vesiculosus*	*F. platycarpus*	*Ulva lactuca*
	per cent	*per cent*	*per cent*
Chemical constituent:			
Ether-soluble fraction	2.3	2.3	0.8
Alcohol-soluble fraction	6.7	8.7	10.6
Cold water-soluble organic matter	13.6	12.1	17.9
Hot water-soluble organic matter	7.8	8.1	10.7
"Hemicelluloses," as reducing sugars	11.2	11.8	11.0
Uronic acid anhydride	27.5	16.1	6.2
"Pentosan," by furfuraldehyde method	28.7	24.5	31.4
"Cellulose," as reducing sugar	4.3	5.4	6.2
"Lignin," or complexes not acted upon by 80 per cent H_2SO_4 in the cold *	5.6	6.2	2.9
Protein	5.4	4.7	12.4
Ash	13.9	14.6	13.6

* This preparation not acted upon by cold 80 per cent H_2SO_4 and boiling 5 per cent H_2SO_4 is not lignin, but a largely carbohydrate-like complex. It is of interest to note that Butler (4) reported the presence of 5.21 per cent " crude fiber " in *F. vesiculosus* and that Schmidt (23) found that 40 per cent of the polyglycuronic acid in *F. serratus* was insoluble in cold 80 per cent H_2SO_4.

tion of the results. The fractions reported as "cellulose" and "lignin" do not necessarily represent the corresponding complexes in higher plants, for considerations similar to those presented above for these constituents in the zoöplankton.

The results of the analyses of the algal materials are also in line with those found by others. These algæ are usually reported (3) to contain from 1.37 to 3.71 per cent nitrogen, 1.50 to 5.21 per cent crude fiber, pointing to a low cellulose and lignin content, and 15.7 to 33.2 per cent ash. The organic complexes are made up largely of carbohydrates, which could be classified with the hemicelluloses, including both true saccharides and polyuronides (8). Although a few specific carbohydrates have been definitely demonstrated (8, 10) in algal material, such as mannitol, arabinose, galactose and methyl pentose or fucose, most of the constituents are still imperfectly described under such names as algin, laminarin, fucoidin, fucin, etc. Some of these complexes represent polyuronides, as in the case of the polymer of mannuronic acid, which is readily isolated from *Fucus* by dilute alkali solutions (1, 11).

DECOMPOSITION OF ZOÖPLANKTON BY MARINE BACTERIA

Our knowledge of the function of bacteria in decomposing the plant and animal residues in the sea is still very limited, the available information being largely based upon speculation rather than upon experimental evidence. It was early recognized that the bacteria active in the decomposition processes in the sea also synthesize new organic complexes in the form of bacterial cell substance. Vernon (17), for example, reported that the largest amount of purification of ocean waters is carried on through bacterial agency; when sea water was kept in the darkness the bacteria removed a large part of the ammonia in the water and synthesized considerable slime; larvæ grown in this water were larger than normal, which led Vernon to conclude that the bacterial cells serve as nutrients for these larvæ. The energy necessary for this bacterial synthesis is no doubt derived from the dissolved organic substances in the water, which have been found (12) to range from 3 mg. of carbon per liter of water in the Baltic Sea to 11–14 mg. in the Kiel Fjord. However, Moore (9) concluded that only a maximum of 1 mg. of organic matter is dissolved in one liter of water. According to Gran and Ruud (5) and Krogh (7), the organic matter in solution is greatly in excess over that present as plankton; this organic matter was looked upon as mainly a waste product, which is not readily available for animal nutrition, and only to a limited extent to bacterial development. Korinek (6) has shown that when fresh water bacteria are placed in the sea they are unable to attack algal residues, while marine bacteria are able to bring about active decomposition of the algal material; however, no quantitative measurements were made. The decomposition of marine algæ added to the soil brought out the fact (15) that the larger the nitrogen content of the algæ the greater is the amount liberated as ammonia during decomposition.

In the following investigations on the decomposition of marine plant and animal residues by bacteria, not only was there an attempt made to study the rate of decomposition and the liberation of nitrogen as ammonia, but also to establish definite relationships between the composition of the substrate and the rate and nature of its decomposition.

For the study of the decomposition of the zoöplankton, 150-cc. portions of fresh sea water were placed in a series of 300-cc. long-necked flasks; another series of flasks received 100-gram portions of fresh marine mud (containing about 50 per cent moisture) and an additional 50 cc. of fresh sea water. Some of the flasks were left as controls, while some received one-gram portions of the air-dry plankton. All the flasks were connected, in the laboratory (16–20° C.), with an aëration apparatus (21), and aërated daily for about one hour. The air, first freed from CO_2, was made to bubble through the liquid of the culture or through the surface layer of water, in the case of the mud cultures, and was then passed through tubes containing standard $Ba(OH)_2$ solution, where the CO_2 liberated in the process of decomposition was absorbed. The experiment was allowed to proceed for 19 days.

TABLE III

Decomposition of zoöplankton by bacteria in sea water and in marine mud *

Medium used	Amount of plankton added	CO_2 liberated		NH_3 liberated
		total	above control	
		mgm. C.	*mgm. C.*	*mgm. N.*
Water	0	5.6	—	0.10
Mud	0	3.2	—	0.25
Water	1 gram	69.7	64.1	40.30
Mud	1 gram	103.9	100.7	38.50

* Period of decomposition—19 days.

At the end of the decomposition period, 2-cc. portions of concentrated H_2SO_4 were added to each flask and the cultures thoroughly aërated for about 1 to 2 hours, so as to liberate the CO_2 absorbed in the water. The flasks were then disconnected and analyzed for ammonia, by distillation with MgO; in the case of the mud cultures, the material was first extracted with N/1 KCl solution, and the ammonia determined in the extract. The cultures contained no nitrite or nitrate. The results of the decomposition of the zoöplankton in the sea water and in the mud are given in Table III, while the course of evolution of CO_2 is illustrated graphically in Figs. 1 and 2. The precipitous rise in the amount of CO_2 at the end of the decomposition period is due to the liberation by the final acid treatment of the CO_2 present in the medium as carbonate and bicarbonate.

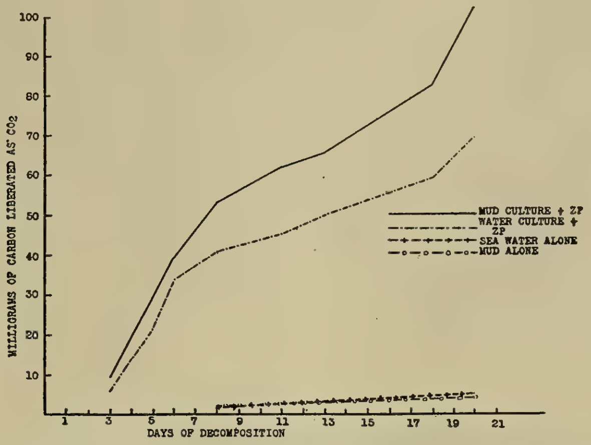

FIG. 1. Accumulated evolution of CO_2 in the course of decomposition of zoöplankton in sea water and in marine mud cultures.

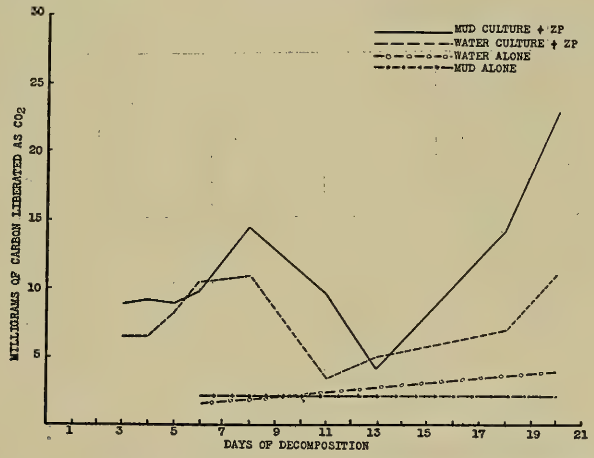

FIG. 2. Daily evolution of CO_2 in the decomposition of zoöplankton in sea water and in marine mud cultures.

The amount of CO_2 liberated from the plankton in the mud as a medium is much greater than in the sea water, while the amount of nitrogen liberated as ammonia is practically the same in both media. Since one gram of the air-dry plankton contained 625 milligrams of organic matter (allowing for moisture and total ash), with about 300 milligrams of total carbon, the liberation in the mud medium of 100 milligrams of carbon as CO_2 within 19 days indicates a very rapid decomposition of the plankton. However, more than a half of the total nitrogen in the plankton has been liberated as ammonia in the same culture and during the same period of decomposition; this points to two definite alternatives: 1. The various chemical constituents of the plankton are not all decomposed at the same rate. 2. More of the plankton is actually decomposed than one is able to measure by the CO_2 evolution, since considerable synthesis of bacterial cell substance takes place in the process of decomposition. The first suggestion is borne out by an examination of the curves in Fig. 2. Although the rate of decomposition had come to a minimum in 11 days, in the case of the sea water medium, and in 13 days, in the case of the mud medium, further decomposition began to take place after that date.

TABLE IV

Decomposition of marine algæ by bacteria in sea water and in marine mud *

Medium used	*Ulva lactuca*			*Fucus vesiculosus*		
	CO_2 liberated		NH_3−N liberated	CO_2 liberated		NH_3−N liberated
	total	above control †		total	above control †	
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. C.*	*mgm. C.*	*mgm. N.*
Water........	89.0	83.4	5.2	16.0	10.4	0.4
Mud..........	105.0	101.8	4.1	89.0	85.8	—

* 1 gram of air-dry material used; 26 days incubation for *Ulva* and 28 days for *Fucus*.

† Water and mud controls were the same as in Table III.

These results suggest the following possibility: some of the constituents of the plankton, probably the proteins, are undergoing active decomposition during an early stage; this is later followed by the decomposition of other constituents of the plankton, probably the chitins and the fatty substances. The specific bacteria capable of attacking these two complexes are probably more active in the mud than in the water. Their decomposition of these substances which are low in nitrogen or are totally free from nitrogen will not only not result in any ammonia liberation but may actually result in ammonia consumption by bacteria for cell synthesis. These two considerations may fully account for the greater decomposition of the plankton in the mud than in the water, as measured by the CO_2 evolution, not accompanied, however, by greater ammonia liberation.

Decomposition of Marine Algæ by Bacteria

The decomposition of the algal material by bacteria was carried out in a manner similar to that of the plankton. The period of decomposition was 26 days for the *Ulva* and 28 days for the *Fucus*. One-gram quantities of the air-dry material of *F. vesiculosus* and *U. lactuca* were added to the flasks containing the water as a medium or the mud and water; the cultures were aërated daily.

The results presented in Table IV and Fig. 3 show that the *Ulva* was decomposed by the bacteria in the water and in the mud more actively than the *Fucus*. In the case of the *Ulva* there was very little difference in the rate of decomposition in the water or in the mud; the *Fucus*, however, showed a very marked difference, since a considerably greater amount of CO_2 was liberated in the mud than in the water cultures. About 4 to 5 milligrams of ammonia-nitrogen were accumulated in the decomposition of the *Ulva*, while there was no ammonia at all or only a mere trace in the *Fucus* cultures. The nitrogen content of the two algæ offers the explanation for this difference. *Ulva* contained 2.56 per cent nitrogen on a total dry, ash and salt-free basis; *Fucus*, however, contained only 1.01 per cent nitrogen, on an ash and salt-free basis.

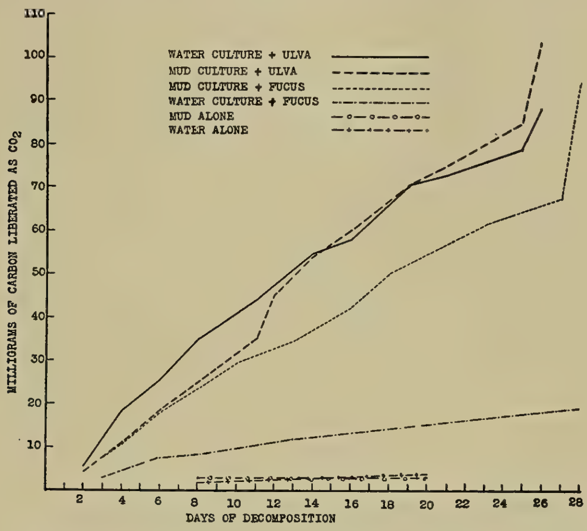

Fig. 3. Course of evolution of CO_2 in the decomposition of marine algæ in sea water and in marine mud.

The difference in the nitrogen content of the two materials has an important effect upon the rapidity and nature of their decomposition. It has been found (23) in the decomposition of plant residues in soils and in composts that if the material contains 1.7 per cent nitrogen, the latter is just sufficient for the active decomposition of the plant substance, without any nitrogen being liberated as ammonia and without any added nitrogen required by the microörganisms. When the plant material contains more than 1.7 per cent nitrogen, the excess will be liberated as ammonia. However, when the nitrogen content is less than 1.7 per cent, some inorganic or available form of nitrogen must be added to enable the bacteria and the fungi to decompose the plant material.

The same law seems to hold true also for the decomposition of algal residues in the sea. There was more than enough nitrogen in the *Ulva* to bring about its rapid decomposition by the bacteria, as shown by the fact that out of 1 gram of the air-dry material, containing 590 milligrams of organic matter with about 265 milligrams carbon, there was liberated nearly 100 milligrams carbon as CO_2 or about 40 per cent of the total, both in the water and in the mud cultures. The *Fucus,* however, containing 827 milligrams of organic matter and about 372 milligrams of carbon in 1 gram of the air-dry material, did not contain sufficient nitrogen for its rapid decomposition by the bacteria.

TABLE V

Influence of addition of available nitrogen upon the decomposition of Fucus vesiculosus in sea water *

Treatment	CO_2 liberation		Inorganic nitrogen left	Inorganic nitrogen disappeared	Numbers of bacteria in 1 cc. of culture
	total	due to added nitrogen			
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. N.*	
2 grams Fucus alone.......	18.9	—	0	0	14,400,000
2 grams Fucus + 50 mgm. $NaNO_3$................	69.5	50.6	0	8.0	70,600,000
2 grams Fucus + 300 mgm. $NaNO_3$................	54.7	35.8	0.5	47.5	31,000,000
2 grams Fucus + 50 mgm. $(NH_4)_2SO_4$..............	47.2	28.3	4.6	5.9	28,600,000

* 2-gram portions of air-dry *Fucus* in 150 cc. of water; decomposition, 28 days.

The greater decomposition of the *Fucus* in the mud than in the sea water may be due to two factors: 1. The organic matter in the mud undergoes some decomposition and the amount of nitrogen liberated in this process may be sufficient for the need of the bacteria, thus enabling them to bring about considerably greater decomposition of the *Fucus*. 2. There is also a possibility that the mud may have a greater abundance of specific bacteria capable of attacking certain specific carbohydrates in the *Fucus*, while the water may be lacking in such bacteria.

In order to study further the factors that are concerned in the decomposition of the nitrogen-poor *Fucus* by marine bacteria, the influence of available nitrogen, both as ammonia and nitrate, has been determined. A series of cultures were prepared using sea water as the medium. All the cultures received 2-gram portions of air-dry, ground material of *Fucus vesiculosus*. The course of decomposition was followed by measuring the evolution of CO_2, in a manner similar to that outlined above. At the end of the incubation period (28 days), the residual nitrogen left as nitrate or as ammonia was determined. The results of this experiment are given in Table V and in Fig. 4.

When no available nitrogen was added to the medium, only a limited amount of *Fucus* underwent decomposition. The addition of a small amount of nitrogen, in the form of an inorganic salt, brought about considerably greater decomposition, the nitrate nitrogen having a more favorable effect than the ammonium salt. An excess of nitrate, however, was less effective than a lower concentration. Where the lower amount of nitrate was added to the cultures, all the nitrogen was consumed by the bacteria bringing about the decomposition of the *Fucus*,

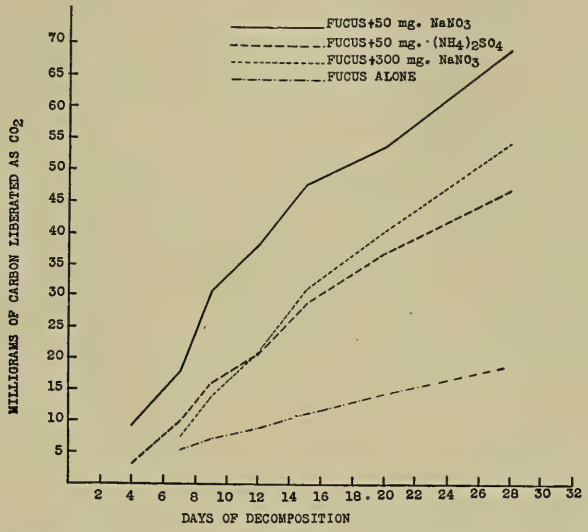

FIG. 4. Influence of available nitrogen upon the evolution of CO_2 in the decomposition of *Fucus vesiculosus* in sea water culture.

for the synthesis of bacterial cell substance. The cultures receiving an excess of nitrate showed a marked loss of the nitrate, through the process of denitrification. The fact that the nitrogen added to the cultures was required by the bacteria for the synthesis of cell substance can be further illustrated by the following considerations: in the decomposition of the algal material, the ratio between the carbon liberated as CO_2 and the nitrogen consumed by the bacteria, for the cultures receiving ammonium salt as a source of nitrogen, was $47.2 : 5.9 = 8 : 1$; the corresponding ratio for the cultures receiving the lower nitrate concentration was $8.7 : 1$. As will be shown later, these ratios are quite normal for the decomposition of carbohydrates by marine bacteria. There was no nitrate left in the cultures receiving the lower amount of nitrate, at the end of the decomposition period, and only a trace of nitrite was present; in the case of the higher nitrate concentration, only a small amount of nitrate was left at the end of the decomposition period, while a considerable amount of nitrite could be demonstrated in the medium.

The above results point definitely to the conclusion that nitrogen in the form of ammonia and nitrate can be used by marine bacteria for synthetic purposes, in the process of decomposition of nitrogen-poor algal material. When a high nitrate concentration is present, the *Fucus* material may be used as a source of energy by denitrifying bacteria for the reduction of the nitrate.

In order to determine what rôle the bacteria play in the decomposition of the *Fucus* material, 1-cc. portions of the various cultures were plated out, at the end of the decomposition period, using glucose-peptone sea water agar. The plates were incubated and the bacterial colonies counted at the end of 4 days. The results (Table V) show that there is a direct parallelism between the development of the bacteria and the liberation of CO_2, in the decomposition of *Fucus* material in sea water medium.

The results of these experiments prove conclusively that the rate of decomposition of marine algæ and the liberation of nitrogen as ammonia depend upon the chemical composition of the algal material. The more rapid decomposition of the *Ulva* than of the *Fucus* is due, aside from any difference in the chemical composition of the two, to the greater nitrogen content of the former. The more limited decomposition of the *Fucus* in the water cultures was due to a lack of available nitrogen, which prevented the development of the bacteria capable of decomposing the algal residues.

On comparing the results of Tables IV and V, one notes that there was a greater decomposition of the *Fucus* in the mud than in the sea water, even with the addition of an available source of nitrogen. This suggests the possibility that nitrogen is only one factor limiting the decomposition of marine algal material in the sea. Possibly the mud contained other elements which are also needed for bacterial growth and which were not present in sufficient amounts in the sea water. The greater decomposition of the *Fucus* material in the mud may also be due to the presence in the latter of specific bacteria, which are able to decompose certain organic constituents of the *Fucus,* while these bacteria are not present in the free water.

Isolation of Cellulose and Hemicellulose-decomposing Bacteria from the Sea

In order to study further the mechanism of the decomposition of algal residues in the sea by bacteria, it was essential to isolate pure cultures of such bacteria and study their action upon certain purified algal constituents. The available information concerning the decomposition of specific constituents of marine plants by bacteria is largely limited to the agar-liquefying bacteria. Beginning with the work of Gran (4) in 1902, there were isolated from the sea a number of organisms capable of liquefying and decomposing agar. In most cases, as in the original form described by Gran, the agar was merely softened and, since no sugar was demonstrated, it is doubtful whether actual decomposition of the agar took place. Other organisms, as the form isolated from the marine mud of the Bahama banks and studied by Waksman and Bavendamm (18), decomposed the agar actively, liberating a large part of the carbon as CO_2; the bacteria, using the agar as a source of energy, were able to synthesize considerable cell substance.

Little is known concerning the occurrence in the sea of organisms capable of decomposing cellulose. Issatchenko (quoted in ref. 13) isolated from the mud of the salt lake Ssaky in Crimea two organisms, one of which decomposed cellulose without forming a pigment (*Bact. cellulosæ album*), while the other colored the paper yellow (*Bact. cellulosæ flavum*). Rubentschik (13) isolated from the salt limans in southern Russia an *Actinomyces* (*A. melanogenes*) capable of slow decomposition of paper. In 1929, Van der Lek (16) demonstrated the occurrence in the sea of *Bact. agar-liquefaciens,* which is capable of attacking cellulose and agar.

The following investigations deal with the occurrence in the sea of bacteria capable of attacking the various chemical constituents of marine residues as well as with the mechanism of the decomposition processes. The carbohydrate constituents were at first selected, due to their predominance in the algal residues. Three methods were employed for the isolation of the bacteria from sea water and from marine bottom material:

1. *The Silica-gel Method.*—Silica gel plates prepared by a method described elsewhere (19) are placed in flowing tap water, so as to bring about the removal of the excess of chlorides by dialysis; the dishes are then transferred to boiled sea water and kept there for about 1 hour; the last step may be repeated, using another portion of freshly boiled sea water, to insure sterility. The dishes are carefully removed from the water, the excess water drained off, and the surface covered with a small amount of powdered $CaCO_3$; for the purpose of isolating cellulose-decomposing bacteria, ground filter paper is placed on the surface of the gel; for the isolation of agar-decomposing organisms, powdered agar is used. A sterile salt solution consisting of 4 grams $NaNO_3$ and 2 grams K_2HPO_4 in 100 cc. of distilled water is then added to the surface of the medium, using 1-cc. portions for the small plates (15 cm. diameter) or 5-cc. portions for the large plates (25 cm. diameter). Plates so prepared were inoculated with fresh sea water, with marine bottom material or with pieces of algal growth.

2. *Enrichment Culture Method.*—This consists in placing 250-cc. portions of fresh sea water, 0.5 gram $NaNO_3$ and 0.1 gram K_2HPO_4, with or without 2-gram portions of $CaCO_3$, into a series of 250-cc. Erlenmeyer flasks. One per cent of cellulose, in the form of ground filter paper, or 1 per cent of dry agar were used as sources of carbohydrate.

3. *The Nutrient Agar Plate.*—This is made up to contain 1 gram of glucose, 1 gram peptone, 0.5 gram K_2HPO_4 and 15 grams agar in 1000 cc. of sea water. Marine mud, plankton tow, or fresh sea water are plated out on this medium, using various dilutions.

The cellulose-decomposing bacteria can be demonstrated most readily by the silica-gel plate method, while the agar-liquefying bacteria are best studied by the nutrient-agar plate method. In the case of the sea water, where only few bacteria are present, the enrichment culture may have to be used first, in order to demonstrate the presence of the specific organisms in the water. The cellulose bacteria developing on the silica gel plates are transferred to test tubes containing 5-cc. portions of sterile liquid medium (2 grams $NaNO_3$, 0.5 gram K_2HPO_4, 12 grams $CaCO_3$ in 1000 cc. sea water), with a strip of paper partly immersed in the liquid and partly above it. The agar-liquefying bacteria can be readily obtained in pure culture by the use of the agar plate; the colonies are picked and transferred to agar slants, and, if necessary, the process may be repeated using various dilutions of the culture. In the case of the cellulose bacteria, however, one must have recourse to the use of very high dilutions and repeated transfers in order to obtain pure cultures; this is true, of course, of the highly specific bacteria, which use cellulose as the only source of energy. When an organism is able to attack both cellulose and agar, its purification does not represent any particular difficulties.

The presence in sea water and in the sea bottom of bacteria capable of attacking cellulose could easily be established. These organisms were found to be particularly abundant in the plankton tow. Attention has already been called (20) to the fact that a definite parallelism was found to exist between the numbers of bacteria and diatoms in the sea water and in the plankton; the suggestion has been made that bacteria live in the free water only to a very limited extent, but their life and activities in the sea are closely associated with the development of the plankton organisms, in addition, of course, to the bacteria inhabiting the sea bottom.

A sample of diatom tow obtained from George's Bank (Station 1335) (20) gave an abundant growth of cellulose-decomposing bacteria. Similar results were obtained when the water and bottom mud from several stations in the Gulf of Maine and George's Bank were used for the inoculation of silica gel plates. It was thus possible to demonstrate the presence in the sea of a number of cellulose-decomposing bacteria, which represent a variety of types, as determined by their morphological, cultural, and biochemical characteristics.

Morphologically, the marine cellulose-decomposing bacteria were found to comprise short rods, spiral-forming organisms, vibrios, and undulating elongated rods with tapering ends, characteristic of the Cytophaga group. One is able to demonstrate without any difficulty the presence in the sea of the three groups of cellulose-decomposing bacteria which have been classified by Winogradsky (24), namely, *Cytophaga* (elongated, flexuous rods), *Cellvibrio* (bent rods with rounded ends), and *Cellfalcicula* (spindle and sickle-shaped). In crude culture, the cellulose-decomposing bacteria were invariably accompanied by numerous protozoa, including flagellates, ciliates and amœbæ, which feed upon the bacteria. All the bacteria were actively motile.

The marine cellulose-decomposing bacteria produce in culture a variety of pigments, namely, pink, orange, yellow, brownish-yellow, or no pigment at all. Most of them are aërobic. Some anaërobic forms were also demonstrated, which were found capable of producing gas actively with cellulose as the only source of energy. These organisms were not isolated, however, in pure culture and were only insufficiently studied. As to the utilization of carbohydrates, some of the cellulose-decomposing bacteria were found to be highly specific, being unable to use any other source of energy, except cellulose. Some attacked not only cellulose but also agar and other hemicelluloses, as will be shown later. Some were able to utilize a variety of sugars.

Agar-liquefying bacteria are even more commonly found in the sea; they are especially abundant in the diatom tow and on marine algæ and their residues, where they seem to find the necessary nutrients for their development. It was pointed out elsewhere that 1 cc. of diatom tow was found to contain 2,100 to 2,500 cells of bacteria capable of liquefying agar; they made up 5.7 to 6.7 per cent of the total number of colonies developing on the plate.

A number of bacteria were isolated, either directly from the plate or after preliminary enrichment culture with cellulose or agar in sea water. Crude cultures of cellulose-decomposing bacteria on the silica gel plate or in enrichment cultures of cellulose in sea water invariably yielded agar-liquefying bacteria. In some cases, these bacteria could decompose both cellulose and agar, while in other cases the agar-liquefying organisms merely accompanied the cellulose-decomposing forms. The bacteria thus isolated were found to belong to several groups varying considerably in their physiological characteristics. No attempt will be made to describe and classify these organisms. Three cultures were selected for a more detailed study of the decomposition of carbohydrates; the cultural and morphological characteristics of only these cultures are presented here.

Culture No. 2 (b) was isolated from the silica gel plate containing cellulose as the only source of energy and $NaNO_3$ as a source of nitrogen. In crude culture it attacked both cellulose and agar and produced gas as a result of the decomposition of the agar. When freshly isolated, it produced lilac colored to pinkish-brown colonies, on the surface of the agar.

Culture No. 3 was also isolated from the same plate as No. 2 (b). It was found to form, however, two types of colonies, one pinkish in color and one colorless. The pinkish culture was later found to be the same as No. 2 (b), and was designated as 2 (a); it also decomposed both cellulose and agar. The second culture could not utilize cellulose, but acted upon agar alone and was designated as No. 3.

Culture No. 15 formed a yellowish to dirty-brown color upon agar and in liquid culture. It did not attack cellulose, but could utilize agar as the exclusive source of energy.

The agar used for these studies was the Bacto-Difco agar. For later studies, specially washed agar was prepared in quantity by the Digestive Ferments Co.

A brief description of these three agar-liquefying bacteria is given here.

Bacterium sp. No. 2. Forms long, slender rods; often filamentous and curved in young cultures. A few motile rods are present. Occasionally there appear in these cultures a few extremely motile individuals, small in size, which seem to be identical with some of those found in Cultures 3 and 15. The organism reduces nitrate, decomposes cellulose, and does not liquefy gelatin.

Bacterium sp. No. 3. Forms very short rods, often coccus-like, with an equal proportion of slightly longer, slender rods scattered throughout the field. A few rods of the same length but much thicker appear with polar bodies. A few actively motile rods, often granular, sometimes having the appearance of two very short rods joined together, are also found. The latter appear more frequently among the non-motile forms. This organism liquefies gelatin, does not decompose cellulose, and does not reduce nitrate.

Bacterium sp. No. 15. Microscopically similar to No. 3, except that it contains more motile individuals and fewer coccus-like forms. Cultural characteristics are the same as those of No. 3.

Decomposition of Various Carbohydrates by Cellulose-decomposing and Agar-liquefying Bacteria

When agar or other constituents of marine algæ are decomposed by agar-liquefying bacteria, the carbohydrates are used by the bacteria chiefly as sources of energy, as well as for synthesis of bacterial cell substance. Due to the fact that agar and the other algal carbohydrates usually contain very little nitrogen, an additional source of available nitrogen, either in organic or in inorganic forms, has to be added to the cultures. This nitrogen is consumed by the bacteria and is transformed into organic nitrogenous compounds which are the constituents of the bacterial cell substance. This is clearly brought out in Table VI, where

Table VI

Decomposition of agar by three marine bacteria. Milligrams of dry material.

Culture	Agar left*	Agar decomposed	CO$_2$ evolved as carbon	Agar left, as sugar†	Agar utilized	NO$_3$–N left	NO$_3$–N consumed	Ratio of carbon liberated as CO$_2$ to nitrogen consumed
Control	498	0	0	245	0	26.4	—	
Bacterium sp. No. 2	167	331	91.6	82	163	14.0	12.4	7.4
Bacterium sp. No. 3	144	354	74.7	71	174	13.2	13.2	5.7
Bacterium sp. No. 15	384	114	49.4	187	58	18.8	7.6	6.5

* The total carbon content of the culture was determined and calculated as agar, allowing 44 per cent carbon in the dry, ash-free agar.

† Residual culture was hydrolyzed by boiling for 1 hour with 2 per cent HCl; the sugar formed on hydrolysis was used as measure of residual agar.

the results of the decomposition of agar and consumption of nitrogen by the three bacteria described above are presented. One hundred-cc. portions of sterile sea water medium containing 0.6 gram air-dry agar and 0.2 gram $NaNO_3$ were inoculated with the bacteria and the cultures incubated for 30 days; the CO_2 liberated in the process of agar decomposition was determined by passing CO_2-free air over the cultures and absorbing the CO_2 in standard $Ba(OH)_2$ solution. The actual amount of

Table VII

Influence of nitrogen source and agar concentration upon the decomposition of agar by marine bacteria

Concentration of agar in medium	Nitrogen source	Agar left, as sugar in hydrolyzed residue				Agar consumed, as hydrolyzed sugar		
		Control	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3
per cent								
0.5	$(NH_4)_2HPO_4$	147	0	0	147	147	147	0
0.5	$(NH_4)_2SO_4$	147	127	142	147	20	5	0
1.0	$NaNO_3$	265	127	81	265	138	184	0
0.5	$NaNO_3$	136	0	0	42	136	136	94

Table VIII

Utilization of mono- and di-saccharides by agar-liquefying bacteria. $NaNO_3$ as source of nitrogen, and 1 per cent carbohydrate.

Nature of sugar	Glucose				Galactose			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar..........	430	439	392	68	395	386	401	68
Sugar consumed........	—	0	38	362	—	0	0	327
NO_3-N left............	15.3	15.5	15.7	6.5	15.8	15.5	16.2	10.2
NO_3-N consumed......	—	0	0	8.8	—	0	0	5.6

Nature of sugar	Mannose				Sucrose *			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar..........	411	417	417	53	452	410	424	214
Sugar consumed	—	0	0	358	—	42	28	238
NO_3-N left............	15.3	14.8	11.6	9.3	14.8	15.3	11.6	9.3
NO_3-N consumed......	—	0.6	3.8	6.1	—	0	3.2	5.5

* Measured as invert sugar.

agar decomposed by the bacteria is greater than can be calculated from the residual carbon in the medium, since the latter includes not only the residual agar, but also certain decomposition products and the bacterial cell substance synthesized.

The nature of the nitrogen in the medium is of considerable influence in the amount of agar decomposed by different organisms, as shown in Table VII. One hundred-milligram portions of the nitrogen source were used in all the cultures. Both nitrate and ammonium salts can be utilized by the agar-liquefying bacteria as sources of nitrogen. However, in the case of the ammonium salt, the nature of the acid radical is of importance: the phosphate is as readily available to the bacteria as the nitrate; the sulfate was either not used at all or only to a very limited extent. The concentration of the agar in the medium is significant, since, in the case of one organism at least (*Bacterium* No. 3), agar was actively attacked when present in 0.5 per cent concentration but not in 1 per cent.

The ability of agar-decomposing bacteria to utilize various lower and higher carbohydrates is brought out in Tables VIII and IX. Only one of the three cultures of bacteria used in this study could assimilate all the three monosaccharides tested, namely *Bacterium* No. 3; this form does not attack cellulose and is a rather weak hemicellulose-decomposing form, as shown in Tables VII and IX. All the three organisms could utilize sucrose, the most active form being the one that could use all three monosaccharides. The two strains of the active cellulose and agar-decomposing bacterium (*Bacterium* No. 2) did not attack galactose or mannose.

As to their ability to decompose polysaccharides, the different organisms were again found to differ markedly from one another. The two strains of No. 2, the most active agar-decomposing organism, also decomposed cellulose actively, as well as starch and mannan; however, only one of the two strains (*2b*) could utilize galactan and inulin. The weak agar-liquefying organism (No. 3), which could attack monosaccharides readily, was also able to decompose most actively starch, mannan, and galactan.

The three agar-liquefying bacteria were thus found to show a very distinct specificity as regards their ability to decompose and utilize various carbohydrates. Culture No. 2 (a) attacked readily cellulose and agar, as well as starch and mannan, and sucrose only to a limited extent; it did not use glucose, galactose, and mannose, nor inulin and galactan. The other strains of this organism (No. 2b) decomposed agar and cellulose most actively, as well as inulin, starch, mannan, galactan, and were also able to make some use of glucose and sucrose. Culture No. 3 could not decompose cellulose; it acted upon agar only to a very limited extent and under certain conditions; it was able to use, however, monosaccharides and disaccharides readily, as well as starch, mannan, galactan, and inulin. These three organisms represent only a few of a large number of bacteria present in the sea and capable of attacking various complex and simple carbohydrates.

Table IX

Decomposition of polysaccharides by agar-liquefying bacteria. NaNo$_3$ as source of nitrogen.

Carbohydrate	Agar				Inulin *				Starch			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm.*	0	0	0	0	0	0	77	0	0	0	327	0
Hydrolyzed carbohydrate, *mgm.* sugar	265	127	81	265	205	204	82	189	476	63	354	0
Carbohydrate consumed, as sugar, *mgm.*	—	138	184	0	—	0	123	16	—	413	122	476
NO_3-N left, *mgm.*	15.3	10.7	9.3	11.8	—	14.8	10.2	14.3	15.8	4.9	11.1	7.8
NO_3-N consumed *mgm.*	—	4.6	6.0	3.5	—	0.5	5.1	1.0	—	10.9	4.7	8.0

Carbohydrate	Mannan				Galactan				Cellulose ‡			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm.*	0	0†	23	0	0	0	0.5	0	488	335	300	495
Hydrolyzed carbohydrate, *mgm.* sugar	367	14	38	0	174	182	131	114	459	270	247	455
Carbohydrate consumed, as *mgm.* sugar	—	353	329	367	—	0	43	60	—	155	190	0
NO_3-N left, *mgm.*	—	6.9	7.4	9.3	—	14.1	9.3	10.2				
NO_3-N consumed, *mgm.*	—	8.4	7.9	6.0	—	1.2	6.0	5.1				

* 0.5 per cent used.

† In this culture practically all the mannan has disappeared, with no reducing sugar left in medium; the duplicate, however, had 255 mgm. reducing sugar left.

‡ These figures do not represent reducing sugar, but residual cellulose.

SUMMARY

1. A study has been made of the proximate chemical composition of zoöplankton and of certain marine algæ, of their decomposition by marine bacteria, and of some of the bacteria capable of attacking certain carbohydrate constituents of marine plant life.

2. The zoöplankton was readily decomposed by bacteria in sea water and in marine mud media, with the result that more than a half of the total nitrogen present in the plankton was liberated as ammonia. Although the ammonia produced in the water and in the mud cultures was practically the same, a much greater amount of CO_2 was liberated as a result of the decomposition of the plankton in the mud than in the water medium.

3. It is suggested that the protein constituents of the zoöplankton decompose alike in the water and in the mud cultures; however, other chemical constituents of the plankton, free from nitrogen, such as certain fats, or low in nitrogen, such as the chitins, seem to undergo more rapid decomposition in the mud than in the water.

4. In the decomposition of algal material by marine bacteria, the chemical composition of the algæ is of prime importance. In the case of *Ulva lactuca,* a green alga containing about 2 per cent nitrogen on a dry, salt-free basis, active decomposition took place both in the sea water and in the marine mud cultures. In the case of the brown alga *Fucus vesiculosus,* however, containing less than 1 per cent total nitrogen, very little decomposition took place in the sea water medium, but the material decomposed very actively in the mud medium.

5. The addition of inorganic sources of nitrogen to the *Fucus* material in the sea water cultures resulted in a much more active decomposition, due to the fact that this nitrogen was required by the bacteria decomposing the *Fucus* constituents for the synthesis of their cell substance. The presence of *Fucus* material in sea water may, therefore, result in a minimum amount of available nitrogen. A definite parallelism was found between the development of the bacteria in the culture and the amount of decomposition of the *Fucus* material, as measured by CO_2 evolution.

6. Nitrate and ammonium salt were used readily as sources of nitrogen in the decomposition of the *Fucus* material by bacteria. The addition of an excess of nitrate to the culture proved to be injurious. This excess nitrate was rapidly destroyed by denitrifying bacteria.

7. The sea is found to harbor an extensive population of bacteria capable of utilizing cellulose and hemicelluloses. These bacteria represent a number of distinct types, varying considerably in their mor-

phological and physiological properties. Some of the bacteria were found capable of attacking both cellulose and agar, while others decomposed either cellulose alone or agar alone. Some of these bacteria are also able to decompose a number of other polysaccharides, as well as various mono- and disaccharides.

LITERATURE

1. Bird, G. M., and P. Haas, 1931. On the Nature of the Cell Wall Constituents of Laminaria spp. Mannuronic acid. *Biochem. Jour.,* **25**: 403.
2. Brandt, K., 1898. Beiträge zur Kenntniss der chemischen Zusammensetzung des Planktons. *Wiss. Meeresunters. Kiel, N. F.,* **3**: 43.
3. Butler, M. R., 1931. Comparison of the Chemical Composition of some Marine Algæ. *Plant Physiol.,* **6**: 295.
4. Gran, H. H., 1902. Studien über Meeresbakterien. II. Ueber die Hydrolyse des Agar-agars durch ein neues Enzym, die Gelase. *Bergens Mus. Aarb.,* No. **2**: 1.
5. Gran, H. H., and B. Ruud, 1926. Untersuchungen über die im Meerwasser gelösten organischen Stoffe und ihr Verhältnis zur Planktonproduktion. *Avhandl., Det Norsk. Vid. Akad. Oslo.* I. *Math. Naturv. Kl.,* No. 6.
6. Kořinek, J., 1926. Über Süsswasserbakterien im Meere. *Centrbl. Bakt.,* Abt. II, **66**: 500.
7. Krogh, A., 1931. Dissolved Substances as Food of Aquatic Organisms. *Rapp. Procès-Verbaux. Cons. Intern. Expl. Mer.,* **75**: 1.
8. Kylin, H., 1915. Untersuchungen über die Biochemie der Meeresalgen. *Zeitschr. physiol. Chem.,* **94**: 337.
9. Moore, B., E. S. Edie, E. Whitley and W. J. Dakin, 1912. The Nutrition and Metabolism of Marine Animals in Relationship to (*a*) Dissolved Organic Matter and (*b*) Particulate Organic Matter of Sea-water. *Biochem. Jour.,* **6**: 255.
10. Müther, A., and B. Tollens, 1904. Ueber die Producte der Hydrolyse von Seetang (Fucus), Laminaria und Carragheen—Moos. *Ber. deut. Chem. Gesell.,* **37**: 298, 306.
11. Nelson, W. L., and L. H. Cretcher, 1929. The Alginic Acid from Macrocystis pyrifera. *Jour. Am. Chem. Soc.,* **51**: 1914.
12. Raben, E., 1905. Über quantitative Bestimmungen von Stickstoffverbindungen im Meerwasser. *Wiss. Meeresunters.,* Abt. Kiel, N. F., **8**: 81–102, 279–287.
   Raben, E., 1910. Ist organisch gebundener Kohlenstoff in nennenswerter Menge im Meerwasser gelöst vorhanden? *Wiss. Meeresunters.,* Abt. Kiel, N. F., **11**: 109–118, 303–320.
   Raben, E., 1914. Ibid. *Wiss. Meeresunters.,* Abt. Kiel, N. F., **16**: 207.
13. Rubentschik, L., 1928. Zur Frage der aeroben Zellulosezersetzung bei hohen Salzkonzentrationen. *Centrbl. Bakt.,* Abt. II, **76**: 305.
14. Schmidt, E., and F. Vocke, 1926. Zur Kenntnis der Polyglykuronsäuren. *Ber. deut. Chem. Gesell.,* **59B**: 1585.
15. Stewart, G. R., 1915. Availability of the Nitrogen in Pacific Coast Kelps. *Jour. Agr. Res.,* **4**: 21.
16. Van der Lek, G. B., 1929. Vibrio agarliquefaciens Gray. *Ned. Tijdschr. Hyg., Microbiol., Serol.,* **3**: 276.
17. Vernon, H. M., 1899. The Relations between Marine Animal and Vegetable Life. *Mitt. Zool. Sta. Neapel.,* **13**: 341.
18. Waksman, S. A., and W. Bavendamm, 1931. On the Decomposition of Agar-agar by an Aerobic Bacterium., *Jour. Bact.,* **22**: 91.
19. Waksman, S. A., and C. Carey, 1926. The Use of the Silica Gel Plate for Demonstrating the Occurrence and Abundance of Cellulose-decomposing Bacteria. *Jour. Bact.,* **12**: 87.

20. Waksman, S. A., H. W. Reuszer, C. Carey, M. Hotchkiss, and C. E. Renn, 1933. Bacteriological Investigations of Sea Water and Marine Bottoms in the Gulf of Maine. *Biol. Bull.,* **64**: 183.

21. Waksman, S. A., and R. L. Starkey, 1924. Microbiological Analysis of Soil as an Index of Soil Fertility. VII. Carbon dioxide evolution. *Soil Sci.,* **17**: 141.

22. Waksman, S. A., and K. R. Stevens, 1930. A System of Proximate Chemical Analysis of Plant Materials. *Jour. Ind. Engin. Chem., Anal. Ed.,* **2**: 167.

23. Waksman, S. A., and F. G. Tenney, 1927. The Composition of Natural Organic Materials and their Decomposition in the Soil. II. Influence of age of plant upon the rapidity and nature of its decomposition—rye plants. *Soil Sci.,* **24**: 317.

24. Winogradsky, S., 1929. Études sur la microbiologie du sol. Sur la dégradation de la cellulose dans le sol. *Ann. Inst. Past.,* **43**: 549.

ON THE PLACENTATION OF THE HARBOR PORPOISE (PHOCÆNA PHOCŒNA (LINNÆUS))

GEORGE B. WISLOCKI

Department of Anatomy, Harvard Medical School

Foreword

The harbor porpoise is one of the smallest members of the order of the Cetacea. Hence its placentation is easier to study than in the larger whales where preservation and dissection are often rendered difficult by the bulk of the tissues. I had the opportunity of obtaining the gravid uterus of a harbor porpoise (*Phocœna phocœna* (Linnæus)) through the generosity of Mr. Harry Raven and Dr. Remington Kellogg. Because of compactness of size and the fact that it is in a rather good state of preservation, this uterus has afforded an excellent opportunity to study placentation in one of the Cetacea.

As far as I have been able to ascertain, the literature on this subject is not extensive and most of it is relatively old. On reviewing the literature and examining my own specimen, I was impressed by a number of points of interest which I consider to be new and sufficiently important to be set forth. In the description of the present specimen I shall not give a detailed account of what has already been observed, but will confine myself as much as possible to what is new. I am presenting a number of drawings and photographs of the specimen which give, I believe, a clearer presentation of the structure and relationships of the uterus and fetal membranes than the majority of the figures in the literature. Because of the difficulty involved in obtaining adequately fixed material, the microscopic structure of the placenta has never heretofore been very clearly demonstrated.

The literature which I have had an opportunity to consult consists of the three papers by Turner (1870, 1871, 1875) on the placentation of *Balænoptera Sibbaldii, Orca gladiator,* and *Monodon monoceros;* one by Chabry and Boulart (1883) on the placentation of *Delphinus delphis;* a short account by Gervais (1883) of a gravid uterus of *Pontoporia Blainvillei;* an excellent paper by Klaatsch (1886) on the placentation of *Phocæna communis;* and the work of Guldberg and Nansen (1894) on the development and structure of whales. I have not had an opportunity to consult the paper of Anderson (1878) on *Platanista* and *Orcella.* A good account of his observations is given in Klaatsch's paper. For the still older literature I have had recourse to the contributions of Turner. In regard to the non-gravid female reproductive tract of whales, I am familiar with the paper of Beauregard and Boulart (1882), as well as the excellent studies by Daudt (1898), Hein (1914), and Meek (1918).

The Fetal Membranes

The fetus in the present specimen appears to be near term, judging from the development of its external form. It measures 92 cm. in length from tip of snout to tip of tail over the curvature of the back; 42 cm. from tip of snout to anus along the abdomen.

The general relationships of the amnion, allantois, chorion, and uterus to one another are shown in Plate I. As in all hitherto described specimens of whales, the fetus lies in the left uterine cornu. Its head is directed towards the distal end of the cornu while its tail is folded around to the right side of the body. A reversed position of the fetus *in utero* has often been observed. As is invariably mentioned, the fetal membranes extend into the right uterine cornu. In my specimen the left ovary is much larger than the right one and contains a large corpus luteum. Both Daudt and Klaatsch found the corpus luteum in the left ovary of gravid specimens of *Phocæna.* Moreover, in several non-gravid *Phocænæ* Daudt noted that the left ovary was somewhat larger than the right. To these observations it may be added that in a non-gravid specimen of the nearly related *Tursiops truncatus* I have noticed the left ovary to be larger than the right. On the contrary, Turner found a corpus luteum in the right ovary in one instance in *Orca.* It appears from these data that the left ovary of whales is the larger and is the usual site of ovulation. Moreover, nidation of the ovum is invariably such that the fetus occupies the left uterine cornu. The left-sided predominance of the ovarian tissue is in keeping with observations made on birds and some other mammals (Riddle). Hein has described characteristic differences in *Monodon* in the folds of the mucosa of the two horns in the non-gravid uterus.

As is always described, the allantoic sac, although extensive, is not so large as the amniotic sac. The amniotic sac does not in my specimen extend into the right cornu, this horn being preëmpted solely by the allantois. In the much younger specimen of *Phocæna* described by Klaatsch, amnion, as well as allantois, extends into the right cornu.

The chorion is a sac completely filling the uterus so that it is bilobed. Interesting is its degree of adherence to the uterus in various areas. In the left cornu the chorion strips away easily from the uterine mucosa. In the right cornu and, roughly speaking, in the entire area of contact of allantois and chorion it does not strip easily but clings tenaciously to the uterine mucosa. Conversely, its easy separation from the uterus corresponds roughly to the area of contact of amnion and chorion. The arrows on the periphery of the uterus in Plate I indicate the zone of transition between adherent and non-adherent chorion. Moreover, the relationship of amnion and allantois to the degree of adherence of the chorion to the endometrium can be correlated approximately with the distribution of the allantoic vessels (Plate II). The allantois is a bilobed bag, half of it applied to the chorion on the mesial surface of the left uterine horn, the other lobe extending into and completely filling the right uterine cornu. The short umbilical cord reaches the chorion on the ventral border of the allantois in the left uterine cornu. There are four umbilical vessels—two arteries and two veins. One artery and a vein leave the cord to follow the line of chorioallantoic fusion towards the pole of the left uterine cornu, while the other artery and vein turn in the opposite direction to follow the zone of chorioallantoic fusion of the opposite lobe of the allantois. The latter artery and vein are somewhat

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DESCRIPTION OF PLATES

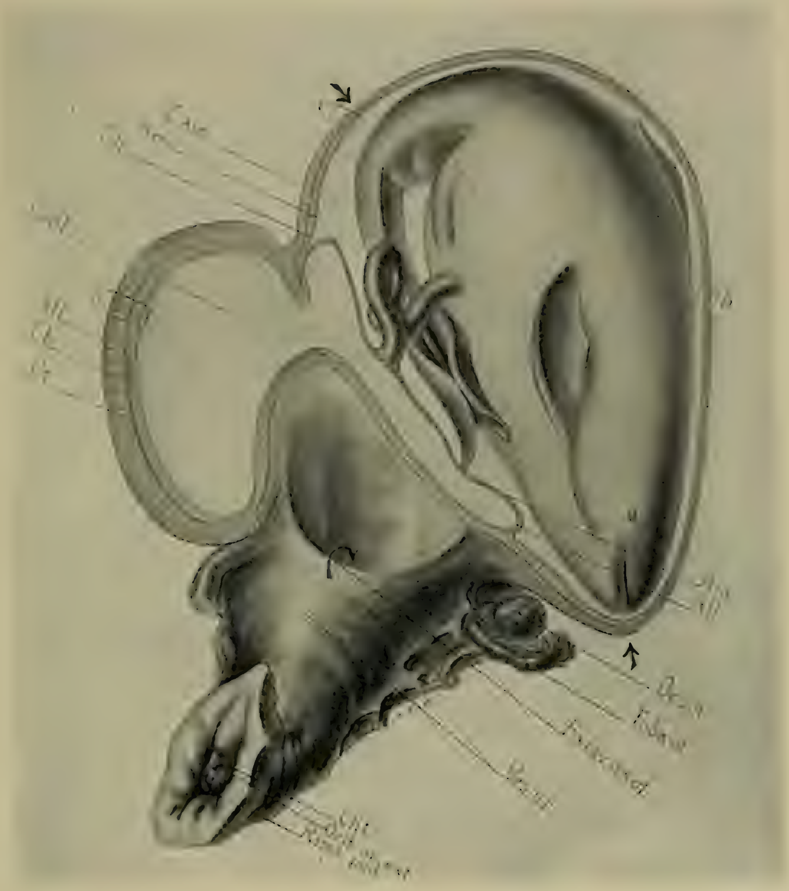
List of Abbreviations

Aa. umb.	Arteriae umbilicales
All.	Allantois
Am.	Amnion
Cav. all.	Cavum allantoidis
Cav. am.	Cavum amnii
Ch.	Chorion
Clit.	Clitoris
D. all.	Ductus allantoideus
Excav. ves. ut.	Excavatio vesico-uterina
Orif. ureth. ext.	Orificium urethrae externum
Ov. sin.	Ovarium sinistrum
Ram. vasa all.	Rami vasorum allantoideorum
Rima pud.	Rima pudendi
Tuba ut.	Tuba uterina
Ureth.	Urethra
Ut.	Uterus
Vag.	Vagina
Ves. ur.	Vesica urinaria
Vv. umb.	Venae umbilicales

Plate I

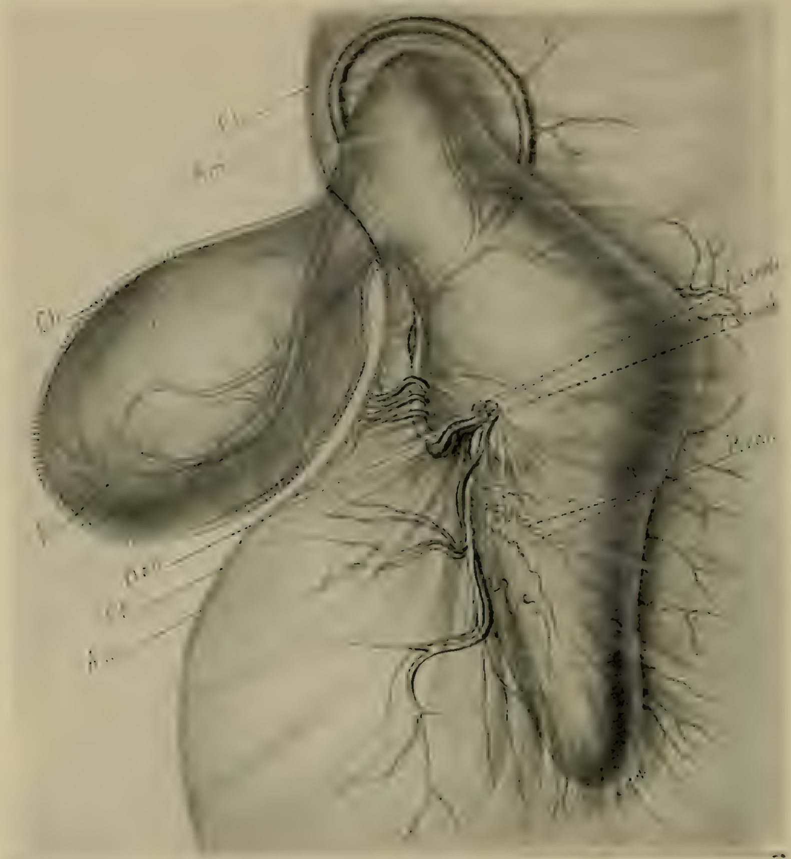
Semi-diagrammatic drawing of the gravid uterus of *Phocœna phocœna.* The fetus lies in the left uterine cornu; the fetal membranes occupy both horns. The relative size and arrangement of amnion and allantois can be seen. The allantochorion is very much thicker than the amniochorion. Three rectangles, marked *a, b, c,* indicate the sites from which blocks of tissue were taken for sectioning. The microscopic appearance of the sections from these localities is shown in Plate IV. The two arrows on the circumference of the uterus at the two poles of the left cornu indicate the transition zone from the relatively thick chorion adherent to the endometrium (allantochorion) to the thin chorion which is readily separable from the endometrium (amniochorion). × 1/7.

PLATE I



larger than the preceding ones. These primary trunks give off numerous branches of succeeding orders which supply the chorion. Now it will be seen that the richest blood supply is destined for the area of

Plate II



Semi-diagrammatic drawing of the allantoic sac to show its shape and the relationship of the umbilical blood-vessels to it. The major distribution of vessels is to the walls of the allantochorion and the neighboring zone of amniochorion. The umbilical vessels are four in number, the two smaller ones running along the inferior medial border of the allantois, the two larger ones coursing upward beneath the allantois to become distributed almost solely to the allantochorion of the right side (left of the figure). The body or left lobe of the allantois (right of the figure) has a free surface facing the observer which is not in contact with the chorion. This surface is avascular excepting a few minute vascular branches (*R. v. all.*). × 1/3.

fusion of allantois and chorion, whereas a lesser number of vessels proceed to the area of contact of chorion and amnion. The further one gets away from the allantois the less numerous the vessels become, and that large area of easily separable contact of amnio-chorion and endometrium in the left cornu, where the uterus is greatly distended, is relatively avascular.

The intimacy of apposition of chorion to the uterine mucosa is found to correspond rather accurately to the blood supply: where the largest vessels run and the richest blood supply appears to exist the chorion is most intimately attached.

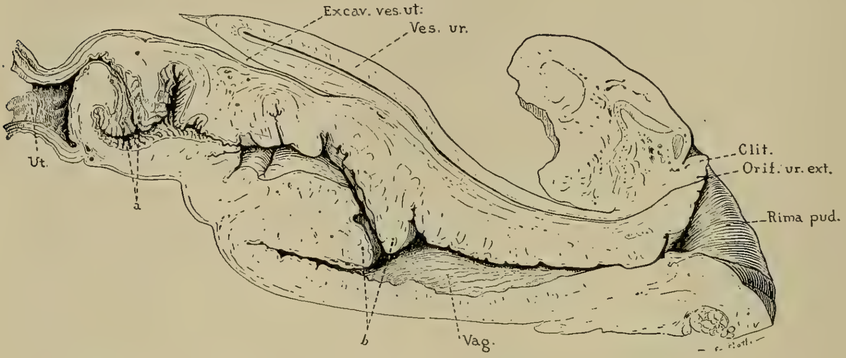
The degree of apposition of chorion to uterine mucosa is, moreover, very definitely related to morphological differences in structure of chorion and mucosa. In the relatively avascular area where the membranes strip easily the chorion is found to consist of a thin membrane which when held to the light is transparent (Plate IV, Fig. 3). Its uterine surface is almost smooth, but on closer examination is found to be covered, nevertheless, by minute villi giving it the texture of thin, rough cloth. The uterine mucosa in this area is correspondingly thin and smooth (Plate IV, Fig. 4). In a typical area of intimate connection of chorion to uterine mucosa, conditions are, on the contrary, quite different (Plate IV, Fig. 1). Traction on the chorion in such an area succeeds not in separating chorion from mucosa, but in either pulling away uterine mucosa from muscularis or the separation of chorionic mesoderm from chorionic villi. Thus in this area chorion and mucosa are rather firmly interlocked. The nature of this interlocking becomes apparent on more careful examination. It can be ascertained that the chorion is covered by branching villi which are placed at brief intervals. These structures fit into small pits with which the mucosa is studded. Separating the chorion from the mucosa is much like attempting to pull the roots of a bush out of the ground. The layer of interdigitated mucosa and chorionic villi is spongy and exceedingly thick compared with the thin chorion and mucosa in the areas of easy separation.

The area of interlocking chorionic villi and uterine mucosa passes by transition to the area where the chorionic villi become shorter and structurally simplified and separation is more easily accomplished (Plate IV, Fig. 2).

This discussion brings up the question of the bare areas or spots of which there is a variety described in various whales by previous writers (Turner, Anderson, Gervais, Klaatsch). Non-villous, membranous areas are described as existing singly or in combination in one or other species of whales in the region of the internal os or at one or both poles of the chorion. In the present specimen there is a bare area only in so far as that general area may be called bare which possesses few and short villi and is relatively avascular. My interpretation of the possible relationship of this area to the blood supply has been outlined above.

A bare area at the internal os is lacking in my specimen, although in this region the chorionic villi can be separated relatively easily from the mucosa. From the concept introduced above of the relationship of blood-supply to a relatively villous portion of the chorion, the bare areas described by previous writers in various species should be investigated again on further material. The difficulty of studying the fetal membranes in larger whales has perhaps obscured the relationships of the chorion to its blood-supply which seem rather apparent here. It should be pointed out also that upon traction artefacts giving the semblance of bare areas may be caused due to the tendency of the chorion, where intimately interlocked with the mucosa, to cleave at the junction of chorionic villi and chorionic mesoderm or between the mucosa and muscularis. The presence of bare spots at the poles of the chorion in several of Turner's specimens led him to point out the similarity of this condition to the type of placenta referred to as *zonary*, as seen typically in carnivores. In the porpoise, however, there is no such resemblance upon which to base a relationship.

Plate III



Drawing of the medial sagittal surface of the lower genital tract. The question of the homologies of the different segments with those of other mammals is of importance. The letters *a* and *b* indicate the structures which might be interpreted as the lips of the external os. In the text reasons are given for considering *b* as the cervical outlet, and the tract between *a* and *b* as a modified and specialized cervical region. × 1/5.

Thus, although the placenta of the porpoise is distinctly a diffuse one, there are morphologically two different areas—one, an area of intimate association of chorionic villi and mucosa which is richly vascularized; the other, an area in which the apposition of chorion to mucosa is less intimate and which is much less vascular. These areas merge imperceptibly into one another.

Microscopic Observations

The microscopic study of the mode of chorionic apposition is of considerable interest. The older writers had no means of determining the minute structure of the chorion, while the more modern investigators were dealing more often with poorly fixed material from larger whales in which the epithelium was largely destroyed by post-mortem changes, or were describing the gross relationships only. Thus in the descriptions and figures of Turner, Klaatsch, and Guldberg and Nansen one is left with considerable doubt as to the finer structure of the chorion and uterine mucosa. In the present specimen the tissues have been sufficiently well fixed to give a fairly adequate picture of the histology.

It was pointed out above that the chorion could be divided roughly into two areas, one of intimate attachment of the chorion to the endometrium, one of extremely loose attachment. It was stated that the area of intimate fusion coincided approximately with the area of the allantochorion; that of less intimate association roughly with the extent of the amniochorion. This separation into two areas is amply borne out by microscopic examination of sections taken from the two regions. Figure 1 is taken from the region of intimate interdigitation of chorion and allantois and shows the broad zone of interpenetration of the spongy endometrium and the villous chorion. Separation of the two membranes is possible only by tearing. In Plate I the place where this section was taken is shown by a rectangle marked *a*. In contrast, Figs. 3 and 4 of Plate IV show the chorion and endometrium in the other region where contact of chorion and endometrium is slight. The site from which the sections were taken is indicated in Plate I by a rectangle marked *b*. It will be seen in the figures that the chorionic villi are entirely absent or stunted in this region and that the endometrium is insignificant in its proportions. Continuing the comparison of these sections with the previous one, it will be observed that the uterine glands are extremely attenuated in contrast to their profusion in the former region. Finally, Fig. 2, Plate IV, shows an intermediate or transitional area between the two regions (rectangle *c*, Plate I). Here the interdigitation and proliferation of chorion and endometrium are less marked than in Fig. 1 taken from the zone of maximum growth.

The main question of interest, however, that can be answered by examination of the present sections is whether the porpoise's diffuse placenta is wholly an epitheliochorial one or is in part a syndesmochorial one. Examination of the present sections indicates that the former is true—that both chorion and uterine mucosa retain everywhere their epithelial covering (Figs. 5 and 6). It can be seen in the area of maximum proliferation of the chorion that both uterine and chorionic epithelium (Fig. 6) are separate and intact layers. The former is composed of flattened, endothelium-like cells with sharply defined boundaries. The chorionic epithelium, on the other hand, is made up of less regularly arranged cells with rather indistinct boundaries suggesting that the epithelium may be in part or wholly syncytial (Fig. 6).

The tufted chorionic villi are applied loosely to the mucosa, excepting at their distal ends which are free, resulting in a considerable separation between the endometrium and the tips of the chorionic villi (Fig. 5). These spaces do not appear to be artefacts, due entirely to shrinkage in fixation. Instead, they have the appearance of being naturally existing spaces filled with embryotrophic material derived from the uterine epithelium or from the abundant uterine glands. The secretion occupying these spaces appears to be formed of amorphous granules. Moreover, the uterine epithelium in this fundic region of the endometrial crypts is more cuboidal in shape than that of the remainder of the crypts where endometrium and chorion are in closer approximation. The secretion which accumulates in these pockets at the tips of the villi is derived presumably in part from the actively secreting uterine glands. The cells constituting the uterine glands appear to be large and swollen as though they were actively secreting. As is well known from the studies of Turner and Klaatsch, several of these uterine glands unite to empty

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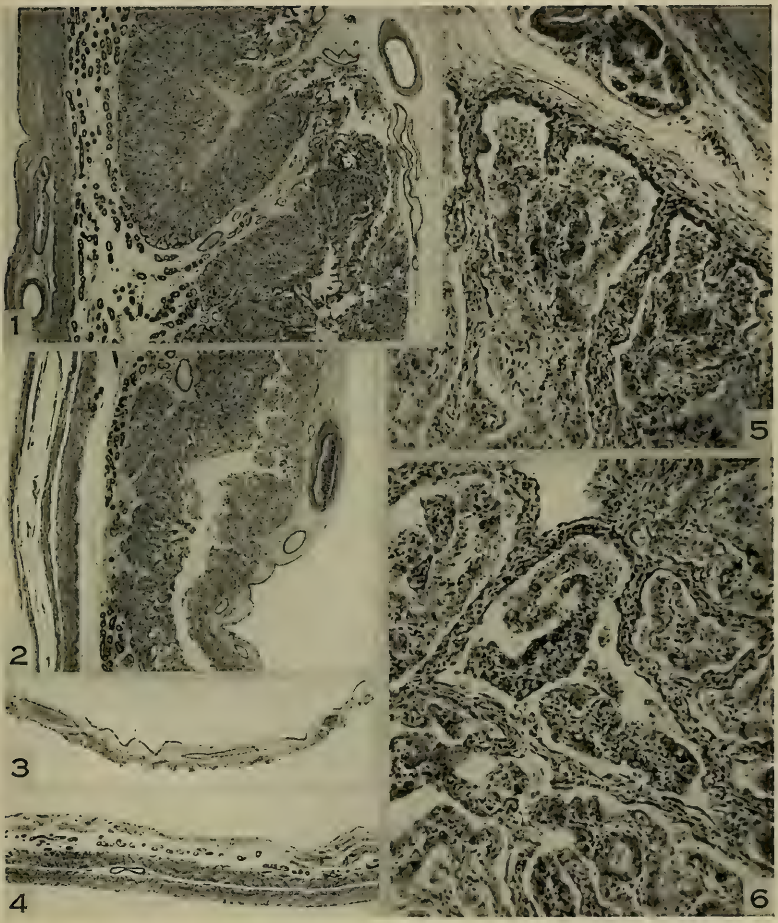
PLATE IV

FIGS. 1 to 4 show typical sections through the uterus and placental membranes from the regions designated *a, b,* and *c* in Plate I. Note that in Fig. 1 (area *a,* Plate I) endometrium and allantochorion are intimately united to constitute a broad placental zone, while in Figs. 3 and 4, which are from the amniochorion and uterine wall respectively of area *b,* Plate I, the chorion and endometrium are simple in structure and consequently are not intimately united. Figure 2 shows an area (*c,* Plate I) of transition midway between areas *a* and *b.* A tendency for separation of chorion and endometrium to occur is apparent. Note that the zone of endometrial glands undergoes a similar simplification from the villous to the non-villous placental area. Figures 1 to 4 are all taken at the same magnification. × 8.

FIG. 5. Higher magnification of a part of Fig. 1. The basal part of the placenta is shown, illustrating the tips of the fetal villi ending free in the endometrial crypts. In the spaces between the tips of the villi and the basal endometrium there is a profuse secretion of uterine milk. The endometrium is composed of a single unbroken layer of cells, cuboidal in the basal region, flattened on the endometrial septa. The covering of the fetal villi is everywhere present, but irregular in thickness, suggesting that the surface layer is syncytial. × 103.

FIG. 6. A photograph of the middle of the placental zone from the area shown under low magnification in Fig. 1. Note the septa or trabeculæ composed of endometrium which enclose spaces or crypts in which the chorionic villi lie. The endometrial trabeculæ are lined by extremely flattened epithelial cells, while the fetal villi are clothed by chorionic epithelium of a syncytial nature and irregularly thickened. × 103.

Plate IV



on the surface by a common outlet which can be seen grossly as minute, whitish flecks designated as areolæ scattered irregularly over the surface of the mucosa when it is viewed stripped away from the chorion. In my experience with the porpoise these are easily seen with the naked eye in the extensive area of mucosa in the left uterine cornu in loose apposition to the chorion. In the area, on the contrary, in which the interlocking of chorion and endometrium is intimate, where the mucosa is thickened and more or less honeycombed for the reception of the chorionic villi, the areolæ are invisible. This, I believe, should not cause surprise for the glandular orifices empty into the deeper, concealed parts of the recesses created by the markedly proliferated endometrium. On the surface actually, after the chorion has been pulled loose, only the crypts are seen which provide for the penetration of the chorionic villi into the spongy endometrium. The areolar areas are invisible. This circumstance has been overlooked by Klaatsch who, I believe, confuses the areolar openings and the mouths of the endometrial crypts which are of a different nature.

In the chorion of my specimen I have looked in vain for corresponding chorionic areolæ or vesicles such as characterize the chorion of the sow which are described as present in specimens of various cetaceans investigated by Turner and Klaatsch. It appears in my specimen, in contrast to the sow, as though the uterine secretion after being discharged from the uterine glands undergoes absorption in the spaces or recesses just described between the tips of the chorionic villi and the funduses of the endometrial crypts, instead of as in the sow, in which small areolar pockets reserved for the absorption of embryotrophe indent the simple chorion, leaving chorionic folds around them, the latter subserving the more intimate union necessary for gaseous exchange. In the porpoise it seems apparent that the tips of chorionic villi dip down into the spongy endometrium to tap the reservoir of maternal embryotrophe situated at the bases of the endometrial trabeculæ, whereas the superficial zone of the endometrium is designed for that more intimate apposition of the chorion, providing for the more direct metabolic exchange.

To continue the discussion of the detailed anatomy of the membranes, Turner and Klaatsch make note of a definite pattern of the chorionic villi, reporting that the villi tend to be arranged or grouped in a linear way often conforming to the course of larger blood-vessels or folds of the chorion. Of this I am able to make out little or nothing in my specimen. When larger areas of the chorion are viewed, one does notice a certain irregularity or patchiness of the velvet-like villi, but this unevenness of height and density does not conform to any definable pattern

The main differences between the height and density of the villi in my specimen are related solely to the two major areas of the chorion emphasized in the previous account.

A few more words should be devoted to the allantois. As has been said, it is a bilobed bag largely in contact with the chorion. The left lobe, however, is fused over about half of its surface with the amnion. The allantois and amnion constitute together a thick transparent membrane separating allantoic from amniotic fluid. In this membrane a few blood-vessels are present which are given off as slender branches from the larger allantoic vessels supplying the chorion (*R. v. all.,* Plate III). In the allantoic fluid are a number of small, free bodies which are brown, irregular in outline, and of the consistency of gum. On the amnion, more particularly that portion fused with the allantois, there are numerous minute, brownish elevations, the carunculæ of previous observers. These structures, which there is no necessity of describing in detail, are especially abundant here as well as on the umbilical cord.

The Umbilical Cord

A few observations of interest have been made on the umbilical cord. A detailed account of structures already well known will not be undertaken. I have paid no particular attention to the epithelial covering of the cord which possesses characteristic epithelial thickenings, so-called carunculæ which have been described in detail for *Phocæna* by Klaatsch and for various other species by Turner.

A cross-section through the middle of the umbilical cord of *Phocæna* reveals two arteries and two veins. Besides these the cross-section shows several duct-like structures (Plate V, Fig. 10). A decision as to the nature of these several ducts offers difficulties, of which Klaatsch was aware. First of all there is a large epithelial-lined duct in the center of the cord which by dissection can be traced in both directions, and is found to communicate with the allantois at one end and with the urachus at the other—evidently the allantoic duct (Fig. 10, *a*). This is lined by cells of variable height: in part they are cuboidal, in other part lower than cuboidal (Fig. 11). Two similar duct-like spaces lined by a similar type of epithelium are found opposite to one another on the periphery of the cord (Fig. 10, *b, c*). In addition to these, several smaller cleft-like spaces lined by epithelium are visible. All of these spaces exhibit the same type of epithelium which suggests that they are related to one another. On tracing the two larger of the clefts by dissection of the cord no final answer as to their relationships is obtained. Nevertheless, in two places they appear definitely to join and to become part of the centrally located allantoic duct. Towards the placenta the two clefts appear to lose themselves in connective tissue. Towards the umbilical ring one of them seemingly joins the allantoic duct. I interpret these clefts as diverticulæ of the urachus or allantoic duct. Near the umbilical ring the allantoic duct is an extensive structure composed of clefts which extend widely around the circumferences of the blood-vessels. It is not unlikely that the aforementioned clefts, which farther out on the cord are seemingly separated from the allantois, are parts of the allantoic duct which are partially separated by septa from the main duct and which communicate with the main duct at one or more points. Klaatsch in his younger specimen found similar evidence of the occurrence of diverticulæ of the allantoic duct. Nothing short of study of serial sections through the cord of *Phocæna* would reveal the exact configuration of the allantoic duct.

In my own material I find no signs of the vitelline duct or remnants of it. In Klaatsch's younger specimens he interpreted certain epithelial structures as the remains of it.

Another striking characteristic of the cord of *Phocæna* mentioned by Klaatsch and especially clearly visible in my own specimen is the presence of bundles of smooth muscle running longitudinally in the stroma of the cord (Fig. 11). Klaatsch interpreted these unusual bundles as remnants of the yolk sac or in part as off-shoots from the muscular tunics of the umbilical vessels. Neither of these explanations accounts, I believe, for the presence of so many stout, rather scattered bundles of muscle fibers. What their nature may be must remain undetermined for the present.

Finally I wish to make a few remarks upon a further interesting observation by Klaatsch. He mentions, as a puzzling feature of the

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PLATE V

FIGS. 7 and 8. Photographs showing at low magnifications the topography of the placenta in the region of area *a*, Plate I. The irregular crypts demarcated by endometrial septa and containing the chorionic villi can be seen. × 47.

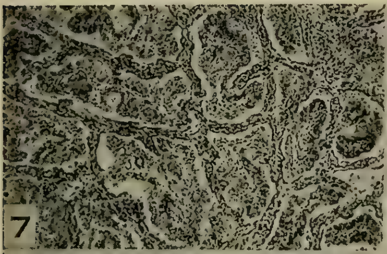
FIG. 9. A view of the stroma of the umbilical cord, showing a number of small blood-vessels by which the stroma is vascularized. × 47.

FIG. 10. Cross-section of the umbilical cord midway between the umbilicus and the allantois, showing the four umbilical vessels. Three clefts, one in the center and two situated peripherally marked *a*, *b*, and *c*, indicate the allantoic duct (*a*) and its diverticulæ (*b* and *c*). The small black dots scattered throughout the stroma of the cord are for the most part small arteries and veins which constitute a vascular bed in the stroma of the cord, similar to the vessels shown under higher magnification in the previous figure. × 8.

FIG. 11. Higher magnification of a part of the umbilical cord adjacent to the allantoic duct showing bundles of smooth muscle fibers scattered through the stroma and bearing no apparent relationship to the musculature of any of the four major umbilical vessels. On the left of the figure two larger, very black areas are small blood-vessels supplying the stroma. × 47.

cord of *Phocæna,* the presence in the stroma of several small arteries and veins, evidently branches of the umbilical arteries and veins. As to the significance of these small vessels in the cord, Klaatsch makes no remarks. In my own specimen these small vessels in the stroma of cord are very distinct and much more abundant than one would have supposed from Klaatsch's observations (Figs. 9 and 10). Indeed the stroma of the cord appears to be richly vascularized at a period near the termination of pregnancy. This vascularization of the cord at such an

Plate V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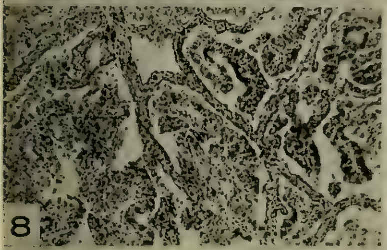
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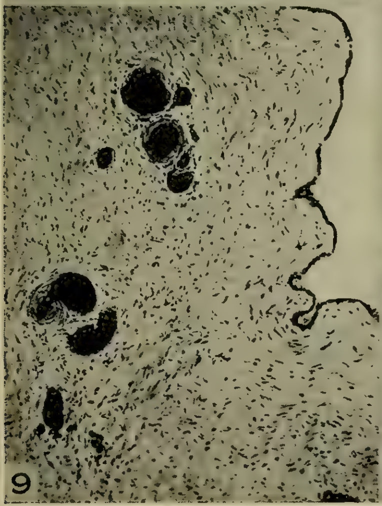
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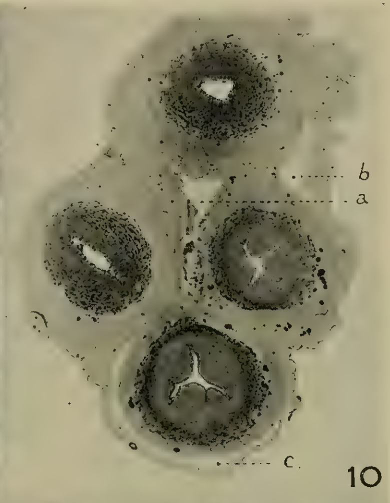

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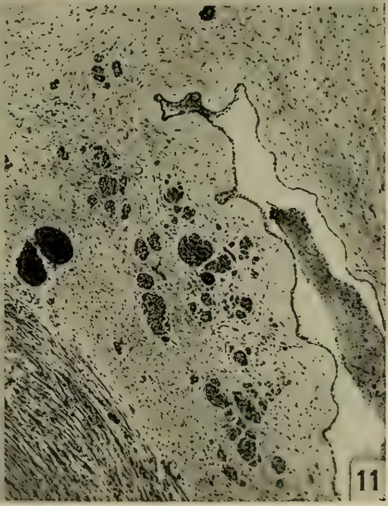
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advanced age is of interest to me because of my observations of the cords of other animals. The umbilical cord of man and other primates is said to have no blood-vessels other than the umbilical arteries and veins; the stroma of the cord is supposedly avascular, possessing no capillaries. Only at the period of blood-vessel formation in the body-stalk is there a vascular plexus in the cord. This period is brief, the vessels of the cord being reduced early to the several major arteries and veins. According to my observations many mammals are quite different from man in that the connective tissue of the fully developed cord at the height of gestation possesses an extensive vascular bed. This blood vascular bed is independent of and has nothing to do with the vitelline blood-supply which in some animals may be extensive and coexistent with it. Grosser (1927) makes no mention of this subject; Jordan (1919), however, describes vascularization of the connective tissue in sections of the umbilical cord of the pig. Other than Klaatsch's remarks on the porpoise and Jordan's observations upon the pig, no further study or discussion of this matter appears to exist in the literature. My first experience in regard to it was with pig fetuses in which, at stages of from 100 to approximately 250 mm. crownrump length, I have been able to inject a rich set of blood-vessels in the stroma of the cord. In cleared specimens I have observed the origin of these vessels in the pig as minute arteries given off at frequent intervals from the umbilical arteries. Small veins similarly join the umbilical veins at short distances apart. The entire cord is vascularized in this manner. On observing sections of the cords of a variety of other mammals I have found evidence of similar vascularization of the tissue composing the cord. Amongst them is the cord of *Phocæna*.

I have made two groups of the animals which I have investigated. I was careful in each instance to take the section from the middle of the cord well away from its attachment to the placenta. Those which have a vascularized stroma of the umbilical cord are:

	Species	Stage examined
Pig	*Sus scrofa*	100–250 mm. C. R.
Hippopotamus	*Hippopotamus sp.?*	Term
Peccary	*Pecari angulatus bangsi*	47 mm. C. R.
Porpoise	*Phocæna phocæna*	Near term
Hyrax	*Proacavia capensis*	147 mm. C. R.
Sloth	*Bradypus griseus griseus*	215 mm.
Agouti	*Dasyprocta punctata isthmica*	126 mm. C. R.
Capybara	*Hydrochœrus capybara*	200 mm. C. R.
Galeopithecus	*Galeopithecus volans*	Middle gestation

Those which have not a vascularized stroma of the cord are:

Tapir	*Tapirella bairdii*	190 mm. C. R.
Galago	*Galagoides demidoffi*	25 mm. C. R.
Marmoset	*Œdipomidas geoffroyi*	47 mm. C. R.
Howling monkey	*Alouatta palliata*	Middle gestation
Spider monkey	*Ateles geoffroyi*	Term
Gibbon	*Hylobates pileatus*	Near term
Chimpanzee	*Pan paniscus satyrus*	Near term

Thus it will be seen that outside of the Order Primates the majority of the animals examined possess well-vascularized umbilical cords and *Phocæna* belongs to the latter category. I hope to publish elsewhere the results of a further study of this matter. What the functional significance of the possession of a vascularized umbilical cord may be remains obscure. It may have some bearing on the mode of formation and changes in quantity of the amniotic fluid.

Besides this capillary blood supply to the umbilical cord of *Phocæna,* the question arises as to whether there are lymphatics in the cord. I mention this because Anderson and Klaatsch describe lymphatics in the cords of their specimens of whales, the latter giving a figure of an endothelial-lined channel which he believes is a lymph-channel. He says in another place in his paper, however, that the epithelial linings of the several structures in the cord were so poorly preserved that he was uncertain as to their morphology. This makes me doubt the accuracy of his observation on the endothelium of the supposed lymphatics. Moreover, he speaks of the anatomical continuity of this lymph channel with tissue spaces. On examination of my own specimen I am led to believe that he and Anderson erred in the identification of lymphatics. I find nothing that resembles true lymphatics. However, without resorting to injection methods to fill lymph vessels, it is impossible to dismiss with certainty the possibility of their existence in a given tissue.

THE GENITAL OUTLET

A sketch is presented of the mid-sagittal view of the genital outlet of the present specimen (Plate III). The topography of the organ is of some interest and deserves a brief discussion. Daudt and Meek both describe the vagina of the porpoise as consisting of two segments—a lower segment that is relatively straight and smooth, and an upper segment of about equal length that is partially subdivided by the presence of several prominent bulging folds. Such an assumption on their part defines the os uteri as a short segment adjacent to the uterus indicated in the present sketch by the letter *a,* Plate III. An alternative interpretation of the homologies of the structures appears to me to bear examination. The os uteri externi could be homologized instead with the bulging lips marked *b* in the drawing, in which case the cervical segment would include all of that segment designated as the upper part of the vagina by Meek and Daudt. In favor of this assumption is the fact that there are well-marked fornices at this point and a rather sharp demarcation between the musculature above and below it, as is shown in the drawing. Moreover, the upper third of this segment, belonging to the vagina according to Meek and Daudt, is clearly glandular as microscopic examination reveals. These glands are abundant and deep, and their secretion completely fills the lumen of the region in question, between *a* and *b,* with a gelatinous mass. This jelly was removed before the sketch was made. Thus the walls of the space immediately caudal to the folds, termed the *spermothecal recess* by Meek, are extremely glandular and the lumen is distended with jelly-like secretion.

To answer the question raised, in view of our almost complete ignorance of the comparative anatomy of the lowermost uterine segment, is impossible. The subject of the homologies of this segment in the mammalian series is one that is almost untouched. By comparison with the human, which is at the moment almost the sole basis for comparison, little help is obtained. Nevertheless, it is known from studies on the macaque (Joachimovits, 1928; Wislocki, 1932) that the cervical segment may be a much more complicated succession of folds than is ordinarily realized. On the other hand, in other animals it may be much less conspicuous than in the human, as for example in the gibbon (Wislocki, 1932). Concerning the question of whether in the Cetacea the elaborate sets of folds at the junction of uterus and vagina should be regarded as a part of the former or of the latter, extreme caution should be exercised until further comparative morphological studies of the subject have been undertaken. In the one instance the cervix would have to be regarded as extremely reduced; in the other event, it would have to be looked upon as a highly developed and specialized structure.

Summary and Conclusions

Examination of a rather well-preserved specimen of one of the smallest species of whales (Phocæna phocœna) demonstrates that near term the placenta is diffuse and is of the epithelio-chorial type. Although diffuse in character, the chorion can be divided at this stage into two rather well-defined areas; the first, an area of intimate interdigitation of branching villi and proliferated endometrium corresponding in the main to the region of allantochorionic fusion; the second, an area of stunted villi and reduced endometrium corresponding roughly to the area of amniochorionic fusion. These areas can be related to some extent to the allantoic blood supply, which is much more profuse in the region of the allantochorion than in the region of the amniochorion.

In spite of the fact that the branching chorionic villi are intimately interlocked in the allantochorionic area with crypts of the uterine mucosa, the endometrium, as well as the chorion, retains its complete integrity by virtue of the survival of intact epithelial coverings. In the case of the endometrium the epithelium is cuboidal in the fundic parts of the endometrial crypts, whereas it is flattened in the outer and more extensive parts of the crypts. The chorionic epithelium, on the contrary, although forming a complete investiture of the villi, is interpreted as being syncytial in character. Thus the placenta is of the epithelio-chorial type, as described in the sow, horse, tapir, hippopotamus, camel, *Manis,* and the lemurs (with the possible exception of *Galago*). The interlocking of chorionic villi and endometrium is, however, much more complex than in the sow, being more like that described in the horse. Whether the attenuated endometrium in the amniochorionic area of the porpoise bears close analogy to the horse, in which widespread degeneration of the endometrium is said to occur in the fourth and fifth months, cannot be ascertained without access to further stages of the porpoise. Be that as it may, the porpoise placenta does not appear to be of the syndesmochorial type which occurs typically in many ungulates with cotyledonary placentæ. It appears inconceivable, however, that at parturition the fetal and maternal tissues, which are intimately interlocked in the area of the allantochorion, are destined to separate without a certain amount of damage to the chorion and endometrium. Either the endometrium must be torn away to some degree or the chorionic villi must become torn off and retained to a certain extent. A separation without trauma to the tissues appears unlikely.

LITERATURE

Anderson, J., 1878. Anatomical and Zoological Researches, Comprising an Account of the Zoölogical Results of the Two Expeditions to Western Yunnan in 1868 and 1875, and a Monograph of the Two Cetacean Genera Platanista and Orcella. London.

Beauregard, H., and R. Boulart, 1882. Recherches sur les appareils genito-urinaires des Balaenides. *Jour. de l'anat. et physiol.,* **18**: 158.

Chabry, L., and R. Boulart, 1883. Note sur un foetus de dauphin et ses membranes. *Jour. de l'anat. et physiol.,* **19**: 572.

Daudt, Wilhelm, 1898. Beiträge zur Kenntnis des Urogenital-apparates der Cetaceen. *Jena. Zeitschr. f. Naturw.,* **32**: 231.

Gervais, H. P., 1883. Sur un utérus gravide de Pontoporia Blainvillei. *Compt. rend. Acad. Sci.,* **97**: 760.

Grosser, O., 1927. Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere. München.

Guldberg, G., and F. Nansen, 1894. On the Development and Structure of the Whale. Bergens Museum. Bergen.

Hein, S. A. Arendsen, 1914. Urogenital System in *Monodon. Verh. Kon. Akad. v. Wetensch. Amsterdam,* 2d Sect., Pt. 8, No. 3, p. 56.

Joachimovits, R., 1928. Studien zur Menstruation; Ovulation; Aufbau und Pathologie des weiblichen Genitales bei Mensch und Affe (*Pithecus fascicularis mordex*). *Biologia generalis,* **4**: 447.

Jordan, H. E., 1919. The Histology of the Umbilical Cord of the Pig, with Special Reference to the Vasculogenic and Hemopoietic Activity of its Extensively Vascularized Connective Tissue. *Am. Jour. Anat.,* **26**: 1.

Klaatsch, H., 1886. Die Eihüllen von Phocæna communis Cuv. *Arch. f. mikr. Anat.,* **26**: 1.

Meek, Alexander, 1918. The Reproductive Organs of Cetacea. *Jour. Anat.,* **52**: 186.

Riddle, Oscar, 1924. Sex in the Right and Left Sides of the Bird's Body. *Proc. Am. Phil. Soc.,* **63**: 152.

Turner, W., 1870. An Account of the Great Finner Whale (*Balænoptera Sibbaldii*) Stranded at Longniddry. *Trans. Roy. Soc. Edinburgh,* **26**: 197.

——, 1871. On the Gravid Uterus and on the Arrangement of the Fœtal Membranes in the Cetacea. *Trans. Roy. Soc. Edinburgh,* **26**: 467.

——, 1875–76. A Further Contribution to the Placentation of the Cetacea (*Monodon monoceros*). *Proc. Roy. Soc. Edinburgh,* **9**: 103.

Wislocki, G. B., 1932. On the Female Reproductive Tract of the Gorilla, with a Comparison of that of other Primates. *Carnegie Inst., Contrib. to Embryol.,* **23**: 163.

POLYMORPHISM AND METHODS OF ASEXUAL REPRODUCTION IN THE ANNELID, DODECACERIA, OF VINEYARD SOUND

EARL A. MARTIN

Department of Anatomy, Cornell University Medical College,
New York City

Remarkable diversity in the reproductive phenomena of the European variety of *Dodecaceria concharum,* an annelid worm, has been shown by the work of Caullery and Mesnil, and Dehorne. The first authors reported three forms which they designated as *A, B,* and *C*. Form *A* was sedentary, parthenogenetic, and viviparous. Form *B* was epitokous, pelagic when mature, and both males and females occurred in approximately equal numbers. Form *C* showed a tendency to become epitokous but was thought to be sedentary when mature. In this form only females were found, and since large mucous glands were present near the gonoducts it was concluded that the form was parthenogenetic and oviparous. Form *A* was most numerous, form *B* occurred in much smaller numbers, and form *C* occurred very rarely.

In 1924 Dehorne reported that a very primitive type of asexual reproduction occurs in form *B* described by the previous workers. In this process the metameres in the middle region of the body become constricted at the annuli and break away into single units. These free metameres regenerate from the anterior surface new head and body segments and from the posterior surface a new anus and body segments. The new individual then becomes a young *Dodecaceria*. More recently, in the fall of 1932, Dehorne has modified this description and reports that the original segment does not actually become incorporated into the new individual but that when the anterior and posterior regenerating ends reach a certain stage they separate at the junction of the new material with the old metamere and by further regeneration form two complete individuals. The original segment may repeat the process of regenerating head and tail portions a second time and these again break away, leaving the old metamere which then probably dies. This breaking up into segments or autotomy of the middle region in the original adult leaves the anterior piece without a tail end and the posterior piece without a head end. Dehorne, 1927, reported that each of these two portions regenerated its missing end and that some of the individuals produced in this manner may give rise to the sexual form *B*.

The *Dodecaceria* of the Woods Hole region were originally described by Leidy and Verrill. In 1855 Leidy recorded a species of annelid found burrowing in dead coral (*Astrangia danae*) at Point Judith which he named *Naraganseta coralii.* This species was subsequently placed in the genus *Dodecaceria* by Verrill. In 1879 Verrill described a species collected in the Bay of Fundy and named it *Heterocirrus fimbriatus.* He reported that this species occurred along with *Dodecaceria concharum* and was related to it. Caullery and Mesnil later called attention to the fact that *Heterocirrus fimbriatus* resembled closely the form *B* of *Dodecaceria concharum* and suggested that it belonged to the genus *Dodecaceria.*

Several years ago the writer found that *Dodecaceria* occurred in abundance in the nodules of encrusting Bryozoa in Vineyard Sound.

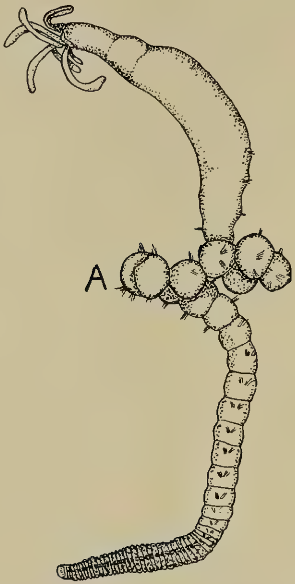


FIG. 1. *Dodecaceria fimbriatus* in asexual reproduction. Region *A* breaking into single metameres by segmental constrictions.

The scant references concerning the American species, together with the interesting phenomena described by Caullery and Mesnil and especially their observation that *Heterocirrus fimbriatus* resembled the form *B* of *Dodecaceria concharum,* led me to begin an investigation of the *Dodecaceria* at Woods Hole. After a preliminary survey intensive investigation was started in July, 1932. At present, collection of material is being made at frequent intervals in order that the life cycle may be determined throughout the year.

Early in this investigation the *Heterocirrus fimbriatus* of Verrill was identified and its resemblance to the form *B* of *Dodecaceria concharum* was found to be so striking that thus far it has been impossible to distinguish differences between them. They appear identical in the development of the eyes, the atrophy of the palps, and the setigerous pattern. A more critical study of the setæ in the two is being made, but on preliminary study similarity extends even to the possession of a tooth at the base of the excavation in the spoon-shaped setæ.

Caullery and Mesnil have described the presence of a gregarine parasite, *Gonospora longissima*, in form *B*. This parasite may be observed as a white mass under the yellow integument. When mature specimens of form *B* are disturbed in the *Lithothamnion* (calcareous algæ) they swim violently, shedding their eggs or sperm, and when empty the gregarines can be seen as long white threads extending out of the gonoducts. The behavior of *Heterocirrus fimbriatus* at maturity is identical with the description of form *B*, and a gregarine parasite is present which also appears to be identical with the *Gonospora longissima* of form *B*. These observations demonstrate that the *Heterocirrus* of Verrill belongs in the genus *Dodecaceria*. It has not been possible to distinguish *fimbriatus* from *concharum*, but since the existence of the

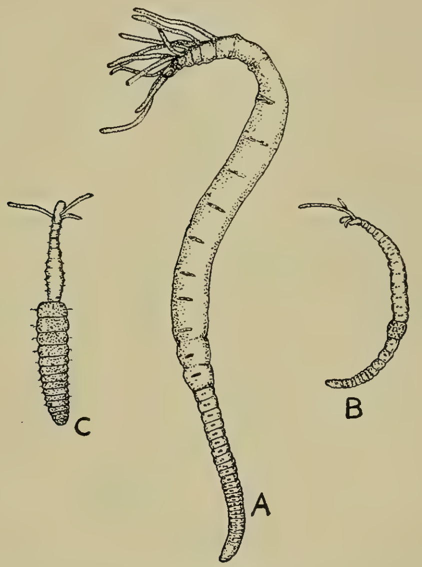


FIG. 2. *A*. Anterior end of worm regenerating a tail after autotomy. *B*. Single segment that has regenerated 12 segments anteriorly and 11 segments posteriorly. *C*. Posterior end of original worm that has regenerated 9 segments anteriorly.

same species on opposite sides of the Atlantic seems improbable, decision is reserved on this point.

Asexual reproduction began in my material during the latter part of the summer. I was unaware of the work of Dehorne and my conclusions thus far have been formed independently of Dehorne's papers. Professor Caullery, during a recent visit to this laboratory, gave me the references to Dehorne's publications.

The phenomenon of asexual reproduction in the American form (Figs. 1 and 2) is essentially the same as described by Dehorne, and those differences that do exist between his observations and mine may be resolved by further study. For example, I have observed, contrary to Dehorne's last report, that single segments may give rise to one individual without further autotomy, in which case the original segment is incorporated into the new individual. This is in line with Dehorne's earlier idea. It has been possible to make this certain since the original segment can be identified in these individuals by a darker band of pigmentation (Fig. 3).

My observations on the further autotomy of the regenerating anterior and posterior ends are not yet complete. I am sure, however, that this phenomenon also occurs in the American form and that it is probably quite extensive since there are relatively few of the young *Dodecaceria* which possess the dark band of pigmentation referred to above. Reliable data on this point can be derived only from newly collected material and not from specimens living in laboratory vessels. It is found

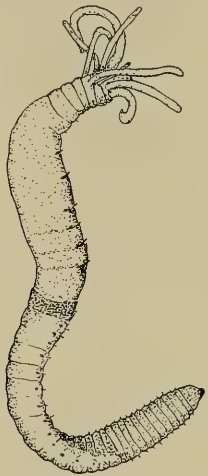


FIG. 3. Young individual derived from a single segment. Note dark band which marks the original metamere.

that when regenerating segments are removed from the bryozoan nodules in which the worms live and are placed in glass dishes, mucus is secreted which accumulates at the junction of the old segment with the regenerating tissue, and there is evidence that this increases the tendency to autotomize at this point.

Since Caullery and Mesnil did not find asexual reproduction in specimens taken from the *Lithothamnion* at Cherbourg and since Dehorne did find both the pelagic form *B* together with asexually reproducing individuals in the marl at Le Portel, Dehorne concluded that the asexual reproduction may have been caused by chemical differences in the material in which the worms were burrowing. The fact that, as my study shows, the phenomenon of asexual reproduction in *Dodecaceria* has a wider distribution than the French workers thought seems to make this conclusion doubtful.

I have recently found indications that the anterior end of the asexual parent produces the epitokous form *B,* thus confirming the report of Dehorne. One specimen, recently found regenerating the posterior end after autotomy, contained the gregarine parasites mentioned above. Another specimen was observed which had developed pelagic setæ and in which the posterior end showed evidences of having recently been regenerated. Further observations on this point are under way.

A careful search has been made for forms resembling *A* and *C,* but thus far there is no evidence that they exist on this side of the Atlantic. Early in my investigation many specimens collected were thought to be form *A,* but it was found that they differed in their morphology from all forms described by European authors. These specimens have been identified as *Dodecaceria coralii.* This species is larger than the European *Dodecaceria,* which never possess as many as 100 segments, while the larger specimens of *Dodecaceria coralii* exceed 100 and may reach as many as 145 segments. This is the most abundant species in Vineyard Sound and the point of chief interest is that it is sedentary throughout life, showing no indications of an epitoke, and that both males and females occur. It is not viviparous.

Caullery and Mesnil, although, as they pointed out, there was some evidence to the contrary, concluded that the forms *A, B,* and *C* were members of one polymorphic species. The close similarity between forms *B* on opposite sides of the Atlantic in both their sexual and asexual phases indicates close relationship. This, together with the fact that the forms *A* and *C* do not occur on this side of the Atlantic, has led the writer to believe that the ensemble *A, B,* and *C* which Caullery and Mesnil have classed as a polymorphic species is actually a group consisting of more than one species. On his recent visit Professor Caullery informed me that work now being done in Europe has led him to the same opinion.

The tentative conclusion concerning the American forms is that two species exist, *Dodecaceria coralii* and *Dodecaceria fimbriatus,* the latter being polymorphic, consisting of sexual and asexual individuals.

The reproductive phenomena displayed by *Dodecaceria* may be arranged in a series in which pelagic epitokous forms occur at one end, producing eggs and sperm, while at the other end reproduction is effected by as primitive a form of asexual reproduction as is known in the phylum. It is interesting to note that the polymorphic form *B* occupies both ends of the series.

Reproductive Series in Dodecaceria

1. Eggs and sperm shed by pelagic epitokous males and females. Form *B, Dodecaceria concharum* and *D. fimbriatus.*

2. Eggs and sperm produced by sedentary atokous males and females that are oviparous. *D. coralii.*

3. Parthenogenetic eggs produced by sedentary epitokous females that are oviparous. Form *C, Dodecaceria concharum.*

4. Parthenogenetic eggs that develop in the cœlom to a larval stage (viviparous). Form *A, Dodecaceria concharum.*

5. Asexual reproduction by separation of single metameres which regenerate complete worms. *D. fimbriatus.*

6. Asexual reproduction by secondary autotomy of regenerating segments. Form *B, Dodecaceria concharum* and *D. fimbriatus.*

Close similarities among the vegetative structures make this a valuable group in which to study the evolution of different methods of reproduction. In spite of wide geographic distribution of these annelids their environmental conditions are similar wherever they occur. The similarity that exists between the biological associations in the *Lithothamnion* at Cherbourg (Caullery and Mesnil) and the association of species in the bryozoan nodules of Vineyard Sound is striking. It is suggested that the uniformity of the environment has decreased the effectiveness of selection on vegetative characters of these worms, while the burrowing habit has increased the effectiveness of selection on possible methods of reproduction in the burrows, thereby avoiding losses incident to pelagic spawning. I infer that asexual reproduction in *Dodecaceria* is a secondary phenomenon.

The identity between *Dodecaceria fimbriatus* and the form *B* of *Dodecaceria concharum* on opposite sides of the Atlantic presents an interesting problem in geographic distribution. If *Dodecaceria fimbriatus* and the form *B* of *Dodecaceria concharum* are different species, which would seem probable, they are so closely related that they must have arisen in a common region. There is small chance of continuity across the abysmal regions of the Atlantic, nor is the duration of life of the pelagic form sufficient for it to be carried any great distance. *Dodecaceria* has been reported as occurring on the piles in Nantucket Harbor, which suggests the possibility that the larva of the pelagic form may live on the encrusted bottoms of ships and in this manner be carried across the Atlantic. However, since *Dodecaceria* is a primitive genus and also since the calcareous materials in which they occur are old geologically, it is probable that these worms have existed in their present habitat for a long period of time. It is planned during the coming summer to learn, if possible, whether there are differences among forms of *Dodecaceria* which range north and south of Cape Cod.

LITERATURE CITED

Caullery, Maurice, and Félix Mesnil, 1898. Les formes epitoques et l'evolution des cirratuliens. Annales de l'Université de Lyon.

Dehorne, Armand, 1924. Multiplication asexuée chez Dodecaceria du Portel par émiettement métamérique ou processus de cténodrilisation. *Compt. rend. Acad. Sci., Paris,* **178**: 143.

Dehorne, Armand, 1927. Le cycle reproducteur annuel de Dodecaceria concharum au Portel. La Schizométamérie. *Compt. rend. Acad. Sci., Paris,* **184**: 547.

Dehorne, Armand, 1932. Nouvelles observations sur la multiplication asexuée d'une Annélide du genre Dodecaceria. *Compt. rend. Acad. Sci., Paris,* **195**: 904.

Leidy, Joseph, 1855. Contributions towards a Knowledge of the Marine Invertebrate Fauna of the Coasts of Rhode Island and New Jersey. *Jour. Acad. Nat. Sci., Phila.,* 2 Ser. No. 3, p. 12.

Verrill, A. E., 1879. Notice of Recent Additions to the Marine Invertebrata of the Northeastern Coast of America, etc. *Proc. U. S. Nat. Mus.,* **2**: 177.

Verrill, A. E., 1881. In New England Annelida. Part I. Historical Sketch with Annotated Lists of the Species Hitherto Recorded. *Trans. Conn. Acad. Arts and Sci.,* **4**: 290.

STUDIES ON THE CILIATES FROM SEA URCHINS

I. General Taxonomy

PHILIP B. A. POWERS

Zoölogical Laboratory, University of Pennsylvania

Introduction

Five (+ 2?) species of ciliates are found infesting the intestinal cæca of *Strongylocentrotus dröbachiensis* (O. F. Müller). In studying these ciliates it was of interest to examine allied species known to be endemic to sea urchins of other localities.

The data growing from these investigations can best be presented in the form of a series of papers of which this is the first. The subsequent papers will (where such studies may be of value) present observations on the detailed morphology of individual or closely allied species. This first report is in the nature of a general survey covering 14 species of ciliates found infesting 3 species of sea urchins.

Materials and Methods

In Table I are shown the various species of sea urchins examined, their source, and the number of species of ciliates comprising their infestation.

In addition to the above, species of *Arbacia* from Cape Cod, the Bay of Naples, and from Beaufort, N.C., were examined for ciliates but with negative results.

When collecting samples of the intestinal contents of sea urchins, it was found advisable to take precautions to prevent any possible contamination of the sample with possible chance mixtures of free-living ciliates. It was found that by rinsing the sea urchin in sterile fresh water and then using clean instruments in subsequent operations, possibilities of chance contamination were eliminated. Observing these necessary precautions, the technique used in this study was essentially that of Lynch (1929): Freshly collected and healthy sea urchins were opened by cutting the test in half with heavy scissors. The perivisceral fluid was drained out and the intestine removed to a clean dish, opened, and its liquid content collected with a sterile pipette and either studied immediately, or preserved in filtered sea water at 10° C., or the material was fixed as smears on coverslips. Material was also preserved in bulk by spurting the intestinal fluid into small flasks of warm fixative.

The most successful fixatives used were Schaudinn's, Bouin's, or Flemming's fluid without acetic acid (F. W. A.). The fixed material was studied in the form of whole mounts or as sections 7 to 10 μ thick. The principal stain employed was iron-alum hæmatoxylin. Mayer's hæmalum, as well as Lynch's (1929) method of using carmine and indulin were found very helpful in confirming observations. Feulgen's nucleal reaction was used as a final check on the nuclear structures of the various forms investigated.

Acknowledgments

This study was initiated in the laboratory of Mr. William Procter of Bar Harbor, Me., during the summer of 1929 and was completed at the University of Pennsylvania. The writer wishes to express his gratitude to all those who have made this study possible and especially to Dr. D. H. Wenrich for his ever ready counsel and helpful criticism.

Table I

Number of species of ciliates comprising infestation of various species of sea urchins examined

Echinoids: Host	Locality	Date of Examination	Nature of Infestation
Strongylocentrotus dröbachiensis (O. F. Müller)	Gulf of St. Lawrence	Sept. 1930	5 species (+2?)
	Roque Is., Me.	Aug. 1930	5 species
	Mt. Desert Is., Me.	July-Aug., 1929–'31	5 species
	Portland, Me.	Nov.-Dec., 1933	4 species
Strongylocentrotus lividus Brd.	Bay of Naples	Aug. 1932	3 species
Echinus esculentus L.	Aberdeen, Scotland	June 1932	———
	Plymouth, England	June 1932	———
Toxopneustes variegatus (Lamarck)	Beaufort, N.C.	April 1931	4 species

The writer wishes also to express his gratitude to the gentlemen directing the laboratories at Naples, Plymouth, Aberdeen, Beaufort, and Woods Hole for their kindness and the abundance of material and equipment supplied him during the course of this investigation.

Ciliates Associated with Sea Urchins

In the following notes, the writer has included all the species encountered, for even in cases where the species has previously been described, the present diagnosis includes points in its description which are here noted for the first time.

In describing the flattened ciliates, the ventral surface is taken to be that one which is most often found in contact with the substratum. When the cytostome is found neither on the ventral nor the dorsal surfaces, but rather on the lateral margin, that margin (edge) is referred to as the oral margin and the opposite side as the aboral margin. This term (oral margin) is also used to designate the side nearest the cytostome. The anterior field is that frontal area, devoid of cilia, which is found on, or bordering, the antero-ventral aboral margin.

Description of Species

Genus CRYPTOCHILIDIUM Schouteden 1906

Kahl (1931) has called attention to the fact that the genus *Cryptochilum* Maupas 1883 is preoccupied by the genus *Cryptochilus* Rafinesque 1815, an hymenopteran; leaving as the next available name, *Cryptochilidium* Schouteden 1906.

Cryptochilidium (*Cryptochilum*) *echini* (Maupas, 1883)

(Plate I. Fig. 3.)

Diagnosis: Average length 83 (45–115)μ, average width 38 (20–55)μ. The body is much flattened, leaf-like with both ends rounded. The region anterior to the middle is exceedingly thin and blade-like, while the region posterior to the middle is somewhat thicker, owing to the food vacuoles which fill this area. The posterior end bears a caudal projection armed with from 4 to 7 bristles, each about 12 to 18 μ in length. Of the two lateral margins, the aboral margin is curved anteriorly while the oral margin is almost straight. The cytostome is located at about the middle of the ventral side of the oral margin. It is particularly conspicuous due to a row of heavy cilia about 12 μ long which are inserted along the length of the right peristomal border. In the buccal cavity, there are found two thin projecting flaps of pellicle which form a pair of non-motile membranes. A small pharynx leads from the buccal chamber to the interior endoplasm. The food vacuoles contain bacteria or fragments of mucosa from sea urchin intestine. A simple contractile vacuole appears posteriorly.

The cytostome is located on the side which appears to be ventral from the fact that the animal uses this surface in contact with the substratum during locomotion. The striations on this ventral surface are parallel with the oral margin and originate in a bare anterior field which runs along the curved aboral margin. The striations on the dorsal surface show a greater curvature than those on the ventral surface because of their origin along the dorsal aboral margin. There are 18 to 20 striations on the ventral as well as on the dorsal surface. The body cilia are about 6 μ long and are not very dense. As the animal rotates while swimming through its medium, the ventral surface is seen to be somewhat concave with the caudal portion bearing its bristles pointing slightly ventrad.

The spherical macronucleus measures about 20 μ in diameter and is found, in the mid-body region, accompanied by a single micronucleus. A number of individuals were observed in fission or undergoing macronuclear re-organization as described by Daïn (1930).

Host: *Strongylocentrotus lividus* from the Bay of Naples. All the specimens examined contained a rather heavy infestation.

Remarks: The size as observed by the writer is in agreement with that given by Daïn (1930). None of the previous workers who have studied this species seems to have made note of the variable number of posterior bristles or the row of heavy cilia along the right margin of the peristome, although the latter are sometimes indicated in the figures of Daïn. In examining cross-sections of the intestine, there have been found a number of cases where the body of *C. echini* is partially embedded in the intestinal mucosa. This indicates the possibility that *C. echini* is a parasite rather than a commensal in relation with its host. It was discovered that these ciliates will exist for 5 or 6 days in sea water after removal from the sea urchin's intestine, thus suggesting the possibility of oral transmission among sea urchins to account for the widespread prevalence of this infestation.

Cryptochilidium (*Cryptochilum*) *bermudense* (Biggar, 1932)

(Plate I. Fig. 1.)

Diagnosis: Average length 150 (90–185)μ, average width 65 (48–82)μ. The body is broad and greatly flattened anteriorly with food vacuoles and cytostome confined to the posterior region, which tends to be somewhat bulbous and bears a caudal projection armed with bristles from 16 to 25 μ in length. The aboral margin is strongly curved anteriorly while the oral margin is straight and contains the cytostome in a depression posterior to the mid-body region. The cytostomal area is marked by an extensive development of the anterior peristomal margin which bears a row of long cilia about its edge. The cytostome opens into a large vestibule or buccal chamber which has hanging from its roof a field of long cilia. The opening to the pharynx is marked by the presence of flaps of pellicle which form a pair of non-vibratile membranes. A long pharyngeal fiber marks off the region filled with large food vacuoles. The endoplasm about this " food basket " is dense and granular. A simple contractile vacuole, sometimes accompanied by small accessory vacuoles, is found in the region posterior to that containing the food vacuoles; while anteriorly is found a large ovoid macronucleus, measuring about 40 × 30 μ, accompanied by a single micronucleus. The region anterior to the macronucleus is clear, vacuolated, and very thin.

The physiologically ventral surface is marked by a greater number of striations than the dorsal surface. These striations are parallel with the oral margin and have their origin in part from a short anterior suture, and in part from the anterior field. This frontal area is unique, for projecting from it (and continuous with the anterior field) are numerous lappets which are exceedingly delicate and devoid of cilia.

Conjugation was not observed, although a number of individuals were found demonstrating phases of macronuclear reorganization.

Host: *Toxopneustes variegatus* from Beaufort, N. C. This ciliate was abundant in all specimens of the host examined.

Remarks: *C. bermudiense* found at Beaufort differs from that described from Bermuda by Biggar (1932) in that it is larger and its macronucleus is also of much greater proportions.

Cryptochilidium (*Cryptochilum*) *echinometris* (Biggar, 1932)

(Plate I. Fig. 4.)

Diagnosis: Average length 122 (80–195)μ, average width 46 (33–70)μ. In shape, the body is oblong and much flattened in the anterior region. The oral margin is almost straight while the aboral margin is slightly curved and bears, anteriorly, an anterior field. The posterior region is bulbous and carries a caudal projection armed with bristles. The cytostome is located very near the oral margin on the dorsal surface, slightly posterior to the middle of the body. The pellicle forming the peristomal region is somewhat thickened and devoid of specialized cilia. There is a field of oral cilia which appears to hang from the roof of the buccal cavity.

The ovoid macronucleus is large, measuring about 20 × 30 μ and is found slightly posterior to the mid-body region, accompanied by a single micronucleus. A simple contractile vacuole is located posteriorly.

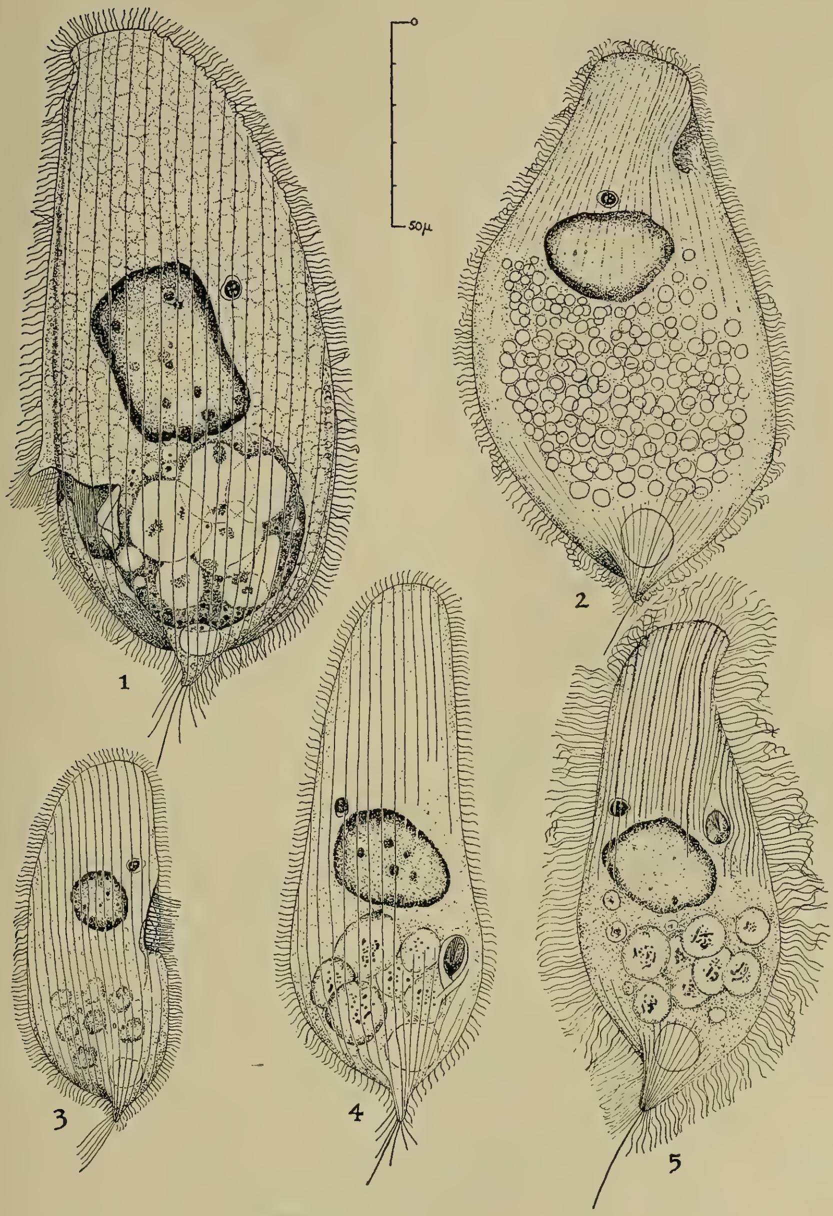
Host: *Toxopneustes variegatus* from Beaufort, N. C. While not very abundant, this species was found in about 50 per cent of the hosts examined.

Remarks: This is undoubtedly the species noted by Bray (1925) under the name of *Cryptochilum echini.*

PLATE I

1. *Cryptochilidium bermudense* (Biggar), as seen from the dorsal surface.
2. *Entodiscus borealis* (Hentschel), dorsal view.
3. *Cryptochilidium echini* (Maupas), ventral view.
4. *Cryptochilidium echinometris* (Biggar), dorsal view.
5. *Cryptochilidium gracile* n. sp., dorsal view.

Plate I



Cryptochilidium gracile n. sp.

(Plate I. Fig. 5.)

Diagnosis: Average length 100 (78–125)μ, average width 45 (40–55)μ. In shape, the body is roughly oblong with the region anterior to the middle of the body greatly flattened, while that part posterior to the mid-body region is bulbous and bears a caudal projection carrying a bristle about 20 μ long. The body is covered with fine, densely placed cilia (12 to 14 μ long) arranged in about 64 rows on the ventral surface and 33 rows on the dorsal surface. Several longitudinal ridges are sometimes noted on the surface of the anterior region. The cytostome is located slightly anterior to the dorsal mid-body region, near the oral margin. The anterior field is ventrad. The peristome is unmarked by specialized cilia, although there are indications of cilia lining the buccal cavity. The ovoid macronucleus, located near the middle of the body, averages 30 × 25 μ in size and is accompanied by a single micronucleus. The large food vacuoles, which fill the posterior region, contain bacteria and bits of algæ.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. The infestation seems universal, varying in degree from one individual to another.

Genus ENTODISCUS Madsen, 1931

Entodiscus borealis (Hentschel, 1924)

(Plate I. Fig. 2.)

Synonymy: *Cryptochilum boreale* Hentschel, 1924.
Entodiscus borealis Madsen, 1931.

Diagnosis: Average length 143 (105–170)μ, average width 87 (60–115)μ. The thickness varies from 25–50 μ. Body is flattened, disc-shaped, narrowing anteriorly, appearing pyriform when seen broadside. The ventral surface is concave while the dorsal surface is slightly convex. The anterior end is thin, narrow, and blunt, with a bald area or anterior field extending over a part of its surface. Posteriorly, the body is broad and rounded, possessing a caudal projection armed with a fine bristle from 15 to 20 μ in length. The body cilia are from 6 to 8 μ long and are densely arranged in parallel rows running antero-posteriorly. There are about 36 rows of cilia on the ventral surface and about 26 rows on the dorsal surface. The cytostome is located in the anterior region near the right lateral (oral) margin of the dorsal surface. The entrance to the buccal cavity is guarded by a pair of labial membranes which are opened only during feeding. From the roof of the buccal cavity there hang a group of cilia from 15 to 20 μ in length.

The cytostome is further marked by the presence of a supporting rod or " stomato-style " which is imbedded in its upper peristomal border. The macronucleus is ovoid, about 28 × 22 μ, and is accompanied by a single micronucleus. The macronucleus appears to rest upon a " nest " of food vacuoles which crowd into the region just posterior to it. The food vacuoles seem to contain bacteria, or mucosal cells from the intestinal lining. A simple contractile vacuole, often associated with numerous accessory vacuoles, is found posteriorly.

Conjugation was not observed, although a number of individuals showed evidence of nuclear reorganization and a few individuals were found undergoing fission.

Hosts: In *Strongylocentrotus dröbachiensis* from the Bay of Fundy, *Entodiscus borealis* occurs in great abundance, while in *Echinus esculentus* from the North Sea, the infestation is very slight, occurring in only about 5–15 per cent of the animals examined from the northern waters as reported by Hentschel (1924). It is possible that *E. borealis* is normally associated with *S. dröbachiensis* but is able to infest *E. esculentus* when the two species of sea urchins inhabit the same locality.

Remarks: This species has many features in common with the genus *Entorhipidium* Lynch; it lacks, however, a definite frontal lobe, and trichocysts; and its cytostomal structures seem to be much more highly developed.

Entodiscus indomitus Madsen, 1931

(Plate II. Fig. 8.)

Diagnosis: Average length 85 (69–120) μ, average width 21 (14–40) μ. The body is greatly elongated with the area anterior to the mid-body region greatly flattened, blade-like, and extremely flexible. The anterior edge is thin, broad, and transparent, while the posterior region ends in a tip bearing a bristle about 16 μ long. The cytostome is located in a small lateral depression at a distance of about one-third of the body length from the anterior end. Out of the cytostome hang two groups of long cilia, an upper and a lower, which seem to be inserted in the back wall of the buccal chamber. A small pharynx leads from the floor of the buccal chamber to the interior endoplasm. The posterior region of the body contains, as a rule, few food vacuoles. A simple contractile vacuole is found, often associated with accessory vacuoles, in the extreme posterior region. The macronucleus is median in position and is always accompanied by a single micronucleus. The body cilia are not particularly dense and are arranged in about 21 parallel rows.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. This species occurs in great abundance but because of its thin body it may be overlooked, for seen edgewise, it is almost invisible.

Remarks: This species undoubtedly has as much in common with the genus *Cryptochilidium* as it has with *Entodiscus.* However, until a detailed morphological study of all the related forms has been completed, the writer will leave this species in the genus to which it was first assigned.

Genus COLPIDIUM Stein, 1860

Colpidium echini Russo, 1914

(Plate II. Fig. 6.)

Diagnosis: Average length 55 (37–64)μ, average width 25 (21–28)μ. The body is cylindrical with both anterior and posterior ends rounded. The cilia are arranged in about 24 rows parallel to the longitudinal axis. The cytostome is obscure, being located in the anterior third of the body as a long narrow groove parallel to the ciliary rows. The right peristomal margin appears to bear a row of long cilia. There seems also to be a group of fine cilia about the entrance to the cytopharynx. A single macronucleus, measuring about 16 × 16 μ accompanied by a micronucleus is found in the region anterior to the middle, usually opposite the cytostome. The micronucleus is usually anterior to the macronucleus. The food vacuoles measure about 7 μ in diameter and fill the endoplasm of the posterior region. A single contractile vacuole is found posteriorly. Individuals were observed conjugating and, in a number of instances, specimens were found demonstrating a type of nuclear reorganization in which the macronucleus had separated into 7 or 8 fragments.

Host: *Strongylocentrotus lividus* from the Bay of Naples. Found, though never abundant, in all specimens of host examined.

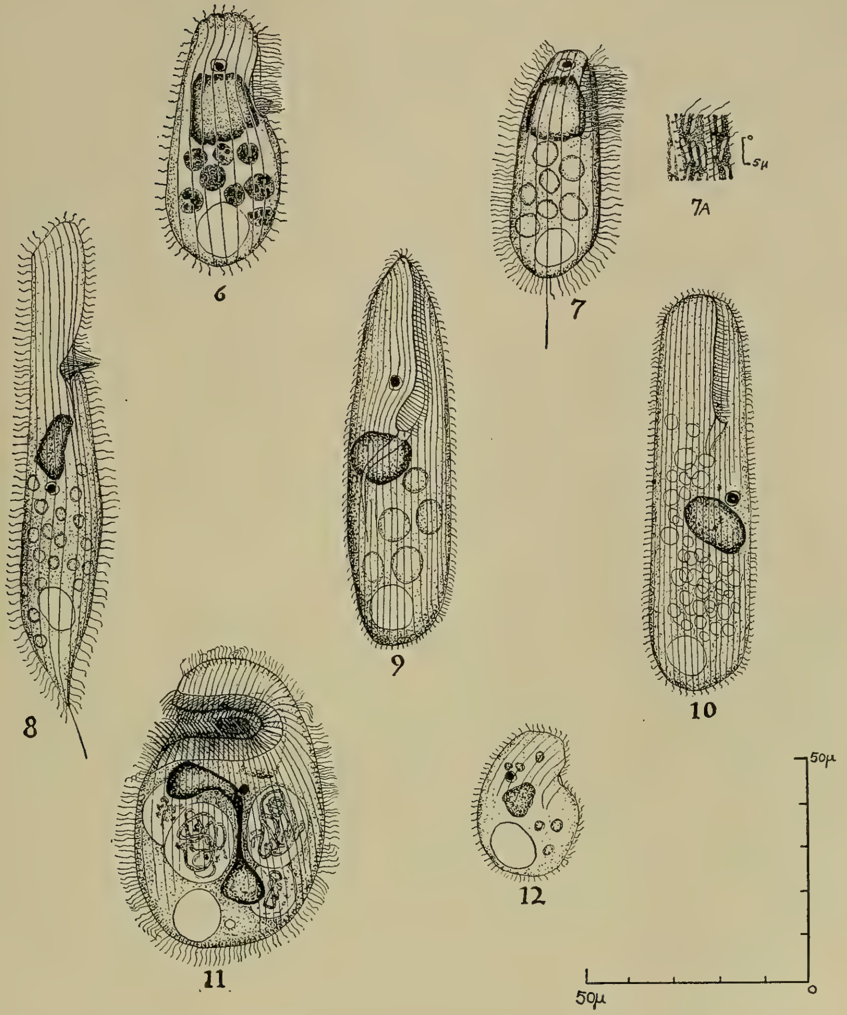
Genus URONEMA Dujardin, 1874

Uronema socialis n. sp.

(Plate II. Fig. 7.)

Diagnosis: Average length 53 (46–70)μ, average width 24 (18–30)μ. The body is cylindrical in shape and of almost constant diameter throughout its length. The anterior field is well marked as a prominent cap on the anterior end devoid of all cilia. The body cilia are about 8 μ long and are arranged in about 14 to 16 rows which run parallel to the longitudinal axis. Orderly arranged groups of bacteria are found covering the pellicle between the rows of cilia (Plate II, Fig. 7*a*). The posterior end is rounded and bears a terminal bristle 15–20 μ long. The cytostome is located in the anterior third of the body and

PLATE II



6. *Colpidium echini* Russo.

7. *Uronema sociale* n. sp., showing general morphology; *7a.* showing a portion of the pellicle illustrating the appearance of the bacteria which apply themselves to its surface.

8. *Entodiscus indomitus* Madsen.

9. *Anophrys echini* Di Mauro, view showing the cytostomal region.

10. *Anophrys vermiformis* n. sp.

11. *Plagiopyla minuta* n. sp., dorsal view.

12. *Colpoda fragilis* n. sp., dorsal view.

occupies a shallow groove, about 4 μ wide, whose right border is marked by a row of long fine cilia. The cytopharynx is obscure and is located at the base of the oral groove. The ovoid macronucleus measures about 15 × 16 μ, and is usually located in the anterior region just opposite the cytostome. A single micronucleus is found often in front of the macronucleus. In the middle and posterior regions food vacuoles, about 6 μ in diameter and filled with bacteria (?), are usually abundant. A simple contractile vacuole is located posteriorly.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. This species occurs in great abundance and while it will live and reproduce in sea water, it is always found in greatest abundance in the alimentary tract of its host. The presence of bacteria on the surface, while visible only under oil immersion, is the most helpful criterion for the identification of this species. These bacteria leave the surface of the pellicle upon the death of *U. socialis.*

Genus ANOPHRYS Cohn 1866

Anophrys echini. Di Mauro, 1904

(Plate II. Fig. 9.)

Diagnosis: Length 40 to 65 μ, diameter 12 to 14 μ. The body is cylindrical and elongated with the posterior end rounded while the anterior is somewhat pointed. The body cilia are short and are arranged in longitudinal rows about 2 μ apart. The cytostome is found at the base of a narrow groove which begins along the margin of the anterior region and extends posteriorly about one-fourth of the body length. The right peristomal border is marked by the presence of a row of cilia which increase in length as they approach the cytostome. Leading from the cytostome is a long and well-marked pharynx. The endoplasm in the region posterior to the cytostome is filled with food vacuoles measuring about 7 μ in diameter. The spherical macronucleus measures about 15 μ in diameter and occupies a median position accompanied by a single micronucleus. A simple contractile vacuole is found in the posterior region. No caudal filament was observed.

Host: *Strongylocentrotus lividus* from the Bay of Naples. The writer found these ciliates very rare among the intestinal contents of *S. lividus.*

Anophrys vermiformis n. sp.

(Plate II. Fig. 10.)

Diagnosis: Average length 85 (68–105) μ, average width 27 (20–35) μ. The body is cylindrical with both anterior and posterior ends rounded. The anterior region of the body is particularly motile and flexible. The cytostome is found in the anterior region at the base of a very shallow and narrow groove. The right peristomal border is marked with a row of cilia which are longer than those of the rest of the body. A short pharynx leads from the base of the oral groove to the interior endoplasm, which is filled posteriorly with food vacuoles. A simple contractile vacuole is found posteriorly. The short body cilia are very fine and are arranged in rows which are about 2 μ apart. The ovoid macronucleus, measuring about 16 × 10 μ, is located near the middle and is accompanied by a single micronucleus.

Host: *Toxopneustes variegatus* from Beaufort, N. C. Occurrence is fairly common.

Genus PLAGIOPYLA Stein, 1860

Plagiopyla minuta n. sp.

(Plate II. Fig. 11.)

Diagnosis: Average length 65 (50–75) μ, average width 40 (36–46) μ, average thickness 30 μ. In shape this ciliate is bluntly oval as seen from the dorsal or ventral surface. The cytostome is marked by a broad transverse groove which extends two-thirds of the distance across the ventral surface in the region anterior to the middle of the body. The cytopharynx opens from the back of this transverse groove and is lined with very short cilia. About the peristome is a wide striated band bearing stiff, heavy cilia. The food vacuoles are very large and are filled with diatoms and strands of algæ. The macronucleus is large and amorphous, fitting its bulk in the space about the nearby food vacuoles. A single contractile vacuole is found posteriorly. The body cilia are fine, measuring about 6 μ in length, and arranged in rows which are about 1 μ apart in the mid-body region. Short trichocysts are embedded in the ectosarc. The dorsal surface is slightly convex and is marked by a striated band about 3 μ wide, which has its origin as a bare area on the oral margin just anterior to the beginning of the peristomal band of cilia.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. Occurrence in about 10 per cent of hosts examined, never over 12 individuals found per host.

Genus COLPODA O. F. Müller, 1786

Colpoda fragilis n. sp.

(Plate II. Fig. 12.)

Diagnosis: Average length 33 (23–40) μ, average width 18 (16–23) μ. Body is much flattened and ovoid. The cytostome is located in a small lateral cleft anterior to the middle. Accompanied by a single micronucleus, the macronucleus measures about 10 μ in diameter and is located slightly anterior to the middle. The body cilia are very short and are arranged in rows about 1.5 μ apart. The endoplasm is usually crowded with food vacuoles and a single contractile vacuole is found posteriorly.

Host: *Toxopneustes variegatus* from Beaufort, N. C. Infestation is abundant when present.

Remarks: Since this species is very sensitive to changes in its environment, observations on the living specimens offered difficulties.

UNDETERMINED SPECIES

There remain two species of ciliates associated with *S. dröbachiensis,* from the Bay of Fundy, whose characteristics could not be determined with sufficient accuracy to permit their identification. One is a member of the genus *Euplotes* and measures about 60 × 35 μ; the other, a member of the genus *Trichodina,* measures about 50 × 30 μ. These two ciliates are found in the alimentary canal of *S. dröbachiensis,* on its exterior and also in the nearby seaweed. Never more than 10 to 15 specimens of either species have been found per host.

DISCUSSION

In regard to the taxonomy of these ciliates, there are at least two alternatives: either one may, by examining cross sections and making a critical study of the cytostomal region, find enough evidence to justify the creation of new genera for several of these superficially similar forms (Plate I, Figs. 1–5), or one may, by a study of the gross microscopic anatomy, group these species, within broad limits, under existing genera. This latter course the writer has chosen for the present, for it will be some time before the detailed morphology of all the forms herein described will be sufficiently known to form the basis of a critical judgment.

In considering these forms which are associated with sea urchins, one sees roughly two groups of ciliates involved. The first group consists of the species which the writer has referred to the genera *Cryptochilidium* and *Entodiscus.* These forms are all elongate, with the region anterior to the cytostome exceedingly attenuate and somewhat like a proboscis. The cytostome is either lateral or nearly so and may possess a peristomal margin of specialized cilia. The buccal chamber is often equipped with a pair of membranes and may contain groups of differentiated cilia. The posterior end bears a caudal tip armed with one or more bristles. The body cilia are usually densely arranged in rows running antero-posteriorly, there being a greater number of striations on the side which is most often in contact with the substratum and hence is termed the physiologically ventral surface. The anterior field is usually found along the anterior margin of this ventral surface. While members of these species will exist for varying lengths of time in sea water of the proper concentration, they nevertheless seem to prefer the environment of the intestinal cæca of their host for active growth and reproduction, and may, therefore, be thought of as being endozoic. The other group consists of the forms referred to the genera *Uronema, Colpidium, Colpoda, Plagiopyla, Euplotes,* and *Trichodina.* These forms are diverse and have many free-living relatives and may be present among the intestinal content of the sea urchin as chance or vagrant ciliates, which, after being engulfed with food, are able to survive in the enteric canal, playing the rôle of scavengers or commensals. One might well argue that this latter group of ciliates, being composed possibly of mere accidental associates, is to be disregarded; however, among all of the possible marine ciliates which might thus be accidentally introduced into the alimentary canal of a sea urchin, it seems significant that these forms described are the only species observed as being involved in this association. Furthermore, it is also of interest to note that of all the members of the Genus *Arbacia* examined, none was found harboring intestinal ciliates, although some species of *Arbacia* are often found in or near localities inhabited by species of *Strongylocentrotus* in which ciliate infestations were found to be universal.

As to the rôle of the first group: they may be considered as commensals with possible pathogenic tendencies. In studying *Cryptochilidium echini* (Maupas) from sectioned material, the writer has observed areas of intestinal mucosa which have been partly penetrated by the ciliates; and from studying *Entodiscus borealis* (Hentschel), there is some slight reason to believe that bits of intestinal mucosa are engulfed by this protozoan, for bodies which closely resemble the nuclei of the cells lining the intestine are often found in the food vacuoles.

The writer now hopes to obtain data on the manner of infestation and of the environmental factors present which allow some of these members of the infestation to be apparently free-living and only occasionally or accidentally associated with their host, while other ciliates of the group seem definitely endo-parasitic. It might also be of interest to discover the factors which bring about such an abundant infestation among the members of the genus *Strongylocentrotus,* while members of the genus *Arbacia* seem devoid of all such ciliate relationships.

SUMMARY

1. Seven species of ciliates are described infesting *Strongylocentrotus dröbachiensis* collected from the Bay of Fundy. This infestation is usually heavy and appears to be universal.

These ciliates are:

Entodiscus borealis (Hentschel)
" *indomitus* Madsen
Cryptochilidium gracile n. sp.
Uronema sociale n. sp.
Plagiopyla minuta n. sp.
Euplotes sp. ?
Trichodina sp. ?

2. Four species of ciliates are described from the intestine of *Toxopneustes variegatus*, collected near Beaufort, N. C.

These ciliates are:

Cryptochilidium bermudense (Biggar)
" *echinometris* (Biggar)
Anophrys vermiformis n. sp.
Colpoda fragilis n. sp.

3. Three species of ciliates are figured from the intestine of *Strongylocentrotus lividus*, taken from the Bay of Naples.

These ciliates are:

Cryptochilidium echini (Maupas)
Anophrys echini Di Mauro
Colpidium echini Russo

4. The relationship of these ciliates with their host is a rather loose one, for the ciliates in question are able to exist for some time in sea water and transmission seems to be orally. The youngest sea urchins examined were lightly infected. There is some evidence to point to a pathogenic rôle on the part of some of the members of the genera *Cryptochilidium* and *Entodiscus*.

LITERATURE CITED

BIGGAR, RUTH BALL, 1932. Studies on Ciliates from Bermuda Sea Urchins (with introduction and notes by D. H. Wenrich). *Jour. Parasit.*, **18**: 252.

BRAY, A. W. L., 1925. Note on the Geographical Distribution of the Protozoan Genus Cryptochilum Maupas. *Science*, N. S., **62**: 589.

DAÏN, LYDIA, 1930. Die Conjugation von Cryptochilum echini Maupas. *Arch. f. Protist.*, **70**: 192.

DI MAURO, S., 1904. Sopra un nuovo infusorio ciliato parassita dello Strongylocentrotus lividus e dello Sphærechinus granularis (Anophrys echini n. sp.). *Boll. Acad. Gioenia Sci. Nat. Catania.*, N. S. fasc., **81**: 13.

HENTSCHEL, C. C., 1924. On a New Ciliate, Cryptochilum boreale, nov. sp., from the Intestine of Echinus esculentus Linn., together with some notes on the Ciliates of Echinoids. *Parasitol.*, **16**: 321.

Kahl, Alfred, 1931. Wimpertiere oder Ciliata (Infusoria). 2 Holotricha s. 388. " Die Tierwelt Deutschlands." Teil 21, Urtiere Protozoa I: 2. verlag von Gustav Fischer, Jena.

Lynch, J. E., 1929. Eine neue Karminmethode für Totalpräparate. *Zeitschr. für Wiss. Mik. für Mikr. Tech.*, **46**: 465.

Lynch, J. E., 1929. Studies on the Ciliates from the Intestine of Strongylocentrotus. I. Entorhipidium, gen. nov. *Univ. Calif. Publ. Zööl.*, **33**: 27.

Lynch, J. E., 1930. Studies on the Ciliates from the Intestine of Strongylocentrotus. II. Lechriopyla mystax, gen. nov., sp. nov. *Univ. Calif. Publ. Zööl.*, **33**: 307.

Madsen, von Holger, 1931. Bemerkungen über einige entozoische und freilebende marine Infusorien der Gattungen Uronema, Cyclidium, Cristigera, Aspidisca und Entodiscus gen. nov. *Zool. Anz.*, **96**: 99.

Maupas, E., 1883. Contribution a l'étude morphologique et anatomique des infusoires ciliés. *Arch. Zool. Expér. et Gén.*, Ser. 2, **1**: 427.

Russo, A., 1914. Specie di ciliata viventi nell'intestino dello Strongylocentrotus lividus Brandt. (Nota Prelim.) *Boll. Accad. Gioenia Sci. Nat. Catania.* Ser. 2, fasc. **32**: 2.

Schouteden, H., 1906. Notes sur quelques infusoires aspirotriches d'eau douce. *Ann. Biol. lacustre* V. 1, p. 440.

STUDIES ON THE CILIATES FROM SEA URCHINS

II. Entodiscus borealis (Hentschel), (Protozoa, Ciliata), Behavior and Morphology

PHILIP B. A. POWERS

Zoölogical Laboratory, University of Pennsylvania

Introduction

The ciliate *Entodiscus borealis* (Hentschel) is one of five species of ciliates found infesting the alimentary tract of *Strongylocentrotus dröbachiensis* (O. F. Müller) taken from the Bay of Fundy. In an effort to find a key to the taxonomic characteristics of these species, some of which are undoubtedly closely related, this ciliate was selected for intensive study because of its large size and apparent simplicity. Sectioned specimens of *E. borealis* revealed many interesting structural details which are reported in this account of its behavior and morphology.

The material used in this study was collected, in the manner described in the preceding paper, from sea urchins taken off Gaspé, Quebec; Mt. Desert Island, and Portland, Maine.

The writer wishes to acknowledge his indebtedness to Dr. D. H. Wenrich through whose helpful guidance and criticism this study was made possible. The writer is also indebted to W. W. Simonton of the Portland High School, who was able to send live sea urchins to Philadelphia where a complete check was made on all the points herein discussed.

Genus ENTODISCUS Madsen 1931

The generic name *Entodiscus* was selected by Madsen to apply to the two flattened holotrichous ciliates which he found inhabiting the intestine of *Strongylocentrotus dröbachiensis* common to the region about Frederikshavn, Denmark. The larger of these two forms appeared to be identical with a ciliate which had already been described under the name of *Cryptochilum boreale* by Hentschel (1924), who pointed out at the time, that further study might justify the creation of a new genus to receive this species.

The writer has examined some of Mr. Hentschel's preparations and feels that the form described in this paper is the same as that species found by him in *Echinus esculentus* from the Shetland Islands; Millport, and Aberdeen, Scotland.

ENTODISCUS BOREALIS (HENTSCHEL)

Observations on Living Material

E. borealis (Fig. 1) is found in great abundance among the liquid contents and food pellets of the sagging loops of the intestinal tract of *Strongylocentrotus dröbachiensis*. Many individuals are found fastened to the walls and in the folds of the intestinal mucosa, while still more are found swimming about in the intestinal fluids which often take on a milky appearance, due to the abundance of ciliates.

E. borealis is very conspicuous due to its large size and the presence of many food vacuoles in its posterior region, which give that region a translucent character. The body shape varies from extremely elongate to oval. In general, the body as seen broadside is pyriform, being greatly flattened as a disc with the dorsal surface slightly arched, while the ventral surface is concave. The anterior region is very thin and narrow, somewhat like a proboscis. The body becomes thicker posteriorly, terminating in a small caudal tip which bears a bristle about 15μ long. The macronucleus consists of a large, homogeneous, egg-shaped mass lying slightly above the middle of the body, seeming to rest in a nest of food vacuoles. The region anterior to the macronucleus is clear and transparent. A large contractile vacuole is found in the posterior region. In one individual whose contractile vacuole was observed during systole, the external vent was seen near the base of the caudal projection. The cytostome is found in a depression near the right anterior margin of the dorsal surface. In living animals, the details of the cytostomal area are obscure; however, in animals which have just died, a number of structures are visible which will be discussed under observations on fixed material.

The body cilia are about 7μ in length, arranged in parallel rows running antero-posteriorly. Because the anterior region of the body is extremely thin and rather narrow, the ciliary rows here are very close together, causing the ciliary movement, which is definitely metachronous, to have the appearance of a series of undulating membranes on the antero-ventral surface. When swimming, these animals rotate on their long axes, moving with steady progress; on the substratum, they move about on their ventral surfaces, often maintaining sufficient suction to defy removal with a pipette. As death ensues, the cilia in the anterior region are the first to cease movement, this cessation of movement proceeding posteriorly until all motion is suspended.

When placed in sea water, these animals will appear normal and will live for various lengths of time, depending upon the temperature and the concentration of the sea water. The writer has kept individuals of *E. borealis* in hanging drop cultures at 7° C., for periods varying from 15 to 23 days. In these hanging drop cultures, there was no reproductive activity, animals seeming merely to exist. After about the second week in a hanging drop, specimens would begin to show a moribund condition, due largely to failure of the contractile vacuole to empty itself.

Because of the ability of this ciliate to maintain itself for various lengths of time outside the body of its host, and furthermore, because this infestation was found to be universal in all specimens of *S. dröbachiensis* examined, even in young sea urchins from 2 to 4 cm. in diameter; it seems likely that the infestation is contracted, orally, through the feeding habits of the developing echinoid. When the liquid passed out of the anus with the fecal pellets is examined, one often finds a number of *E. borealis,* suggesting that the infestation is perpetuated by the passage of these ciliates from the alimentary tract of one sea urchin to that of another, without any intervening cystic stages.

Of all the living specimens examined, only three ciliates were observed reproducing by fission, although many individuals were observed with their macronucleus separated into two or more parts, suggesting stages in which the macronucleus was undergoing reorganization.

Observations on Fixed Material

Measurements of fifty individuals gave the following dimensions: average length 143 (105–170)μ, average width 87 (60–115)μ; the thickness of the posterior region varied from 25–50μ.

When animals are killed with fumes of iodine or osmic acid, the cilia stand out at right angles to the pellicle and may easily be measured. The cilia vary in length from 7 to 11μ, and are arranged in about 70

EXPLANATIONS OF FIGURES 1 AND 2

1. *Entodiscus borealis* (Hentschel). Dorsal view.

2. Camera lucida drawings from cross-sections of *E. borealis.*

A. View of anterior field showing the crowding together of the ciliary rows on the ventral surface.

B. A section passing through the anterior region. Note the basal granules of the oral cilia (*bas. gra'*).

C. Section through the anterior region of the buccal cavity.

D. Section showing the ventral pellicle of the mid-body region.

E. Showing dorsal pellicle of mid-body region.

F. Portion of pellicle from mid-ventral region. Note delicate longitudinal pellicular fibers running parallel to the rows of basal granules.

ant. fib., anterior fibril of the neuromotor system; *ant. field,* anterior field; *ant. peri.,* anterior peristome; *bas. gra.,* basal granules; *buc. cav.,* buccal cavity; *cil. root.,* ciliary rootlets; *dor. sur.,* dorsal surface; *li. cyt.,* lips of cytostome; *lon. pell.,* longitudinal pellicular thickening; *stom.,* stomatostyle; *ven. sur.,* ventral surface.

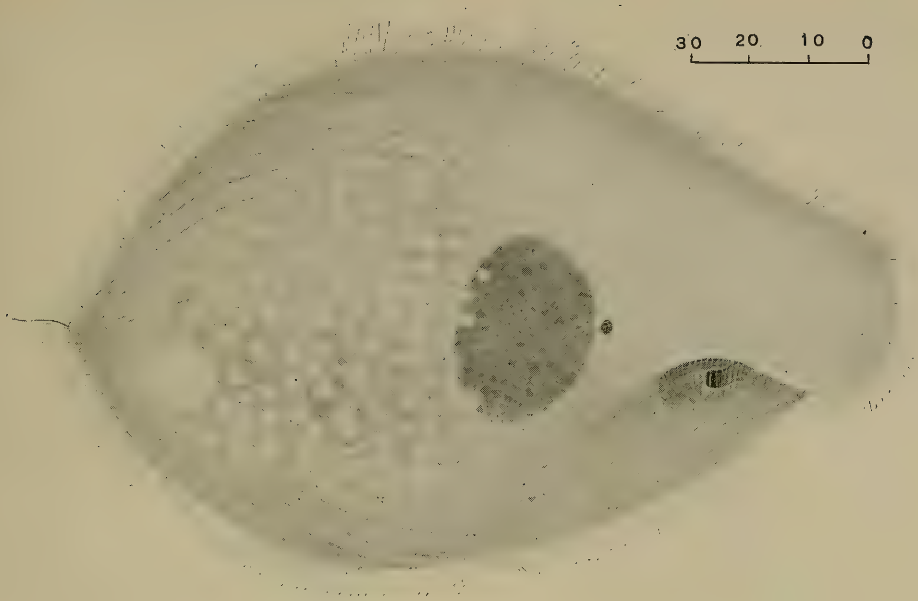


FIG. 1

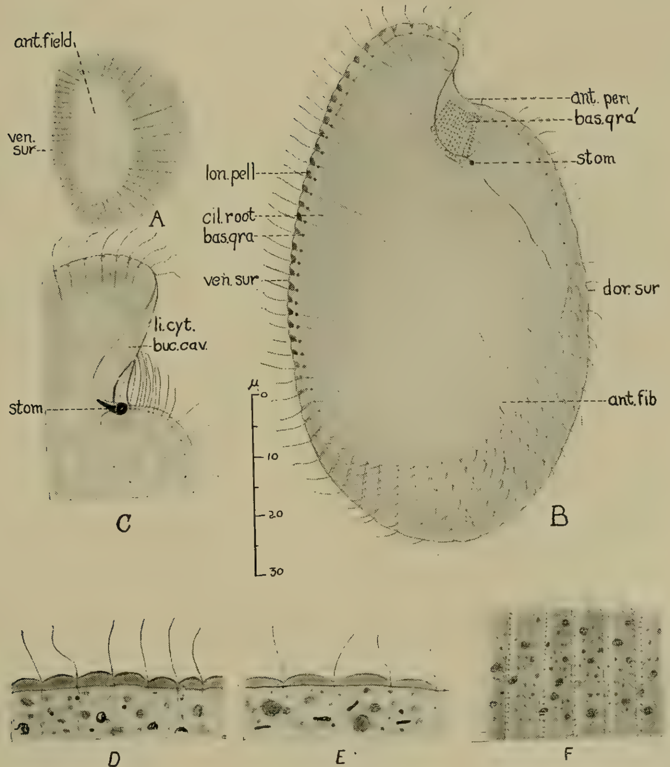

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FIG. 2

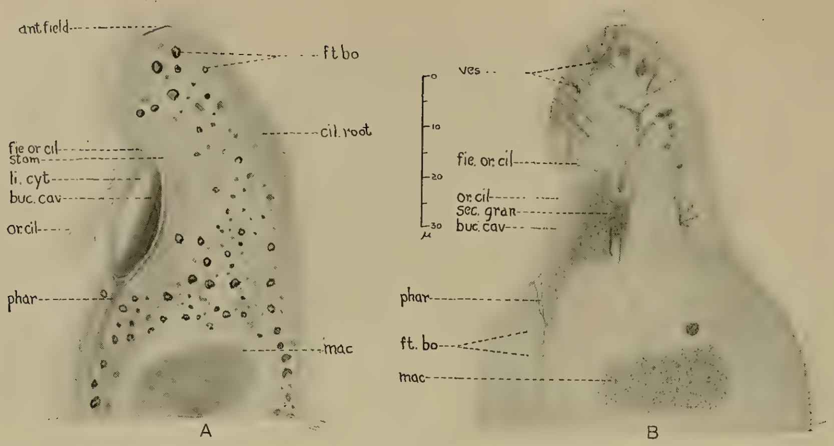
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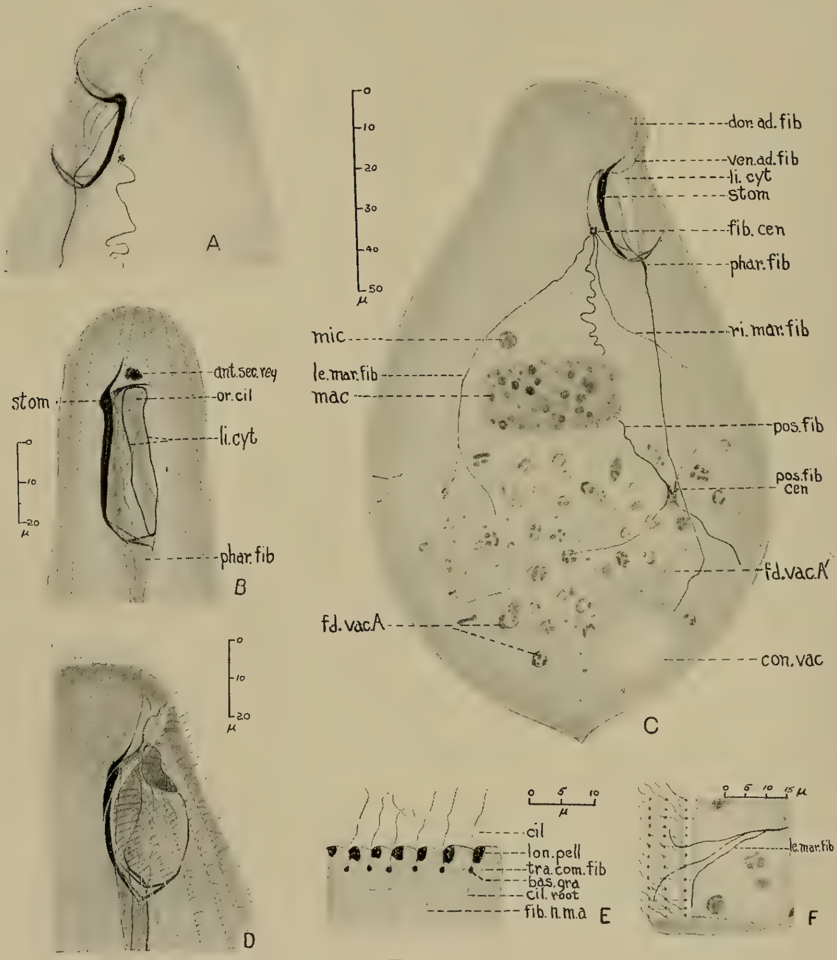
FIG. 3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fib. cen
phar. fib
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FIG. 5

longitudinal rows, each of which lies in a shallow groove, forming a distinct pattern which can readily be seen in the living animals. These rows are much closer together on the ventral surface than they are on the dorsum; there being about 36 rows ventrally and some 24 dorsally. The origin of the ciliary rows is from a bare region on the anterior margin (Fig. 2, *A, ant. field*). This anterior field seems in part comparable to that described for *Entorhipidium echini* by Lynch (1929). In sections of specimens fixed in Champy's, the pellicle of this anterior field is shown to be denser than that of the body surface (Fig. 3, *A*).

The pellicle of the ventral surface is about twice as thick as that of the dorsal surface, and is highly differentiated in its anterior region due to the conspicuous longitudinal pellicular fibrils accompanying each ciliary row (Fig. 2, *B, lon. pell.*). These fibrils, which in the posterior region are very delicate (Fig. 2, *F*), are here very heavy and seem to be either of the nature of a structural "exoskeleton," to protect this region which is most often in contact with the substratum; or they may be of a contractile nature serving as myonemes, explaining in part the mechanism which gives the anterior body region its mobility and allows the creation of the force by which these animals are enabled to hold fast to any smooth surface.

EXPLANATIONS OF FIGURES 3 AND 5

3. Camera lucida drawings of longitudinal sections through cytostomal region showing structures visible after fixation in osmic acid mixtures:

A. Fixed in Champy-Kull. Note the numerous fat droplets.

B. Fixed in Flemming's without acetic (F. W. A.). Note the arrangement of secretory (?) vesicles in the anterior region.

ant. field, anterior field; *buc. cav.,* buccal cavity; *cil. root,* ciliary rootlet; *fie or. cil.,* field of oral cilia; *fa. bo.,* fat bodies or droplets; *li. cyt.,* lips of cytostome; *mac.,* macronucleus; *or. cil.,* oral cilia; *phar.,* pharynx; *sec. gra.,* secretory granules; *stom.,* stomatostyle; *ves.,* secretory vesicles.

5. Details of neuromotor system of *E. borealis.*

A. Cytostomal area as seen through the ventral surface.

B. Camera lucida drawing of cytostome viewed *en face* showing lips fully separated.

C. A complete reconstruction of the neuromotor system as viewed from the dorsal surface.

D. View showing cytostome only partly open.

E. and *F.* Portions of the ventral ectoplasm showing the method by which the body cilia are associated with the fibrils of the neuromotor system.

ant. sec. reg., anterior secretory region; *bas. gra.,* basal granules; *cil.,* cilia; *cil. root.,* ciliary rootlet; *con. vac.,* contractile vacuole; *dor. ad. fib.,* dorsal adoral fiber; *fd. vac. A* and *A',* food vacuoles; *fib. cen.,* fibrillar center; *fib. n. m. a.,* fibril of the neuromotor apparatus; *le. mar. fib.,* left marginal fiber; *li. cyt.,* lips of cytostome; *mac.,* macronucleus; *mic.,* micronucleus; *phar. fib.,* pharyngeal fiber; *pos. fib.,* posterior fiber; *pos. fib. cen.,* posterior fibrillar center; *ri. mar. fib.,* right marginal fiber; *stom.,* stomatostyle; *tra. com. fib.,* transverse commissural fibril.

Just beneath the pellicle along the ciliary rows are the basal granules. These are integrated by means of delicate transverse fibrils (Fig. 5, *E* and *F, tra. com. fib.*), forming a network which is interpreted as a part of the neuromotor system to be discussed later.

The cilia of the ventral surface, particularly of the anterior region, have long ciliary rootlets which penetrate into the endoplasm for about 6–12μ (Fig. 2, *B, cil. root.*). These are very dense in the anterior region and often stain deeply.

No trichocysts were found in the whole mounts or in the sectioned material.

The anterior region is thin, somewhat like a blunt proboscis, with a C-shaped cytostomal groove formed by the downward curve of the right dorsal margin as a slight frontal lobe; making the cytostome partly on the dorsal surface rather than the edge (Figs. 1 and 5, *B* and *D*).

Viewed *en face,* the cytostome, as it appears in the sectioned material, lies in a depression formed by the overhanging frontal lobe. The peristome is formed, in part, by the right margin of this frontal lobe, whose inner surface is the right wall of the buccal cavity. The cilia about this peristomal ridge are from 6–12μ in length and are not much heavier than the body cilia (Fig. 5, *D*). The buccal cavity is guarded by two membranous lips (Fig. 5, *B* and *C, li. cyt.*), which open by separating longitudinally. These lips seem to open along their entire length only during such times as the animal is feeding, being closed or partly so the rest of the time. The lips of the cytostome appear to be formed by a continuation of the delicate membrane lining the buccal cavity. There is found in the roof of the oral depression, just back of the lips of the cytostome where the depression is greatest, an area or field composed of long cilia (Fig. 3, *or. cil.*). These oral cilia measure from 15 to 20μ in length, extending the entire length of the cytostomal region. Judging from the number of basal granules found in this region (Fig. 2, *B, bas. gra'*), some 75 to 100 of these long cilia are inserted in the thickened portion of the membrane (Fig. 3, *fie. or cil.*) which lines the roof of the buccal cavity.

Embedded in the border of the right peristome is a curved supporting rod whose anterior end is forked, and whose slender posterior end terminates among a group of rods which encircle the anterior portion of the pharynx (Fig. 5, *stom.*). This rod appears to form a support for the right wall of the buccal cavity and will be discussed later as a part of the fibrillar system. Because of its obvious nature as a structural element associated with the cytostome, it has been designated the *stomatostyle.*

The pharynx appears to be a definite structure passing downward from the floor of the buccal cavity to disappear near the posterior border of the macronucleus (Fig. 3, *phar.*).

The cytoplasm surrounding the buccal cavity shows two areas which at times are found to be filled with granules suggesting regions associated with the secretion of digestive enzymes. The anterior secretory region (Fig. 5, *B, ant. sec. reg.*) is found just above the thickened pellicle which bears the field of oral cilia. This structure has always been found well defined, as though inclosed by a thin membrane. The larger region of secretory granules is found concentrated near the fibrillar center extending downward along the pharynx (Fig. 3, *B, sec. gran.*).

In the endoplasm are found a number of interesting structures whose demonstration depends largely upon the nature of the fixative used. With the use of Schaudinn's or Bouin's fixative the neuromotor apparatus, nuclear structures, and food vacuoles filling the posterior region are preserved. With the use of osmic acid in the various modifications of F. W. A. (Flemming's without acetic acid) or Champy-Kull technique, there are demonstrated an entirely different set of structures consisting of the secretory regions, fat droplets, and other structures of a lipoidal nature.

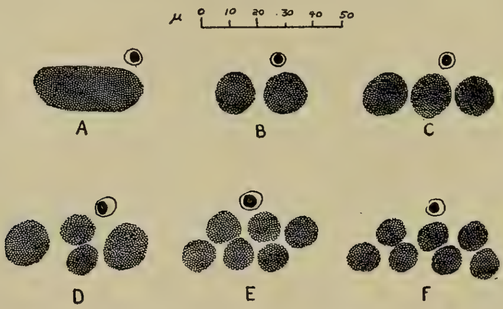
The food vacuoles which usually fill the posterior region of the body, and which are clearly visible in the living organism, appear in sectioned material (fixed in Bouin's) to be of two types: type *A,* those which stain vigorously like nuclear material, and represent newly formed food vacuoles in which the process of assimilation has just begun; and type *A',* vacuoles which stain faintly, usually with a basic dye, and represent the old food vacuoles whose contents have been assimilated (Fig. 5, *C, fd. vac. A* and *A'*). The food vacuoles of dividing individuals are small and homogeneous, showing that their contents have been assimilated.

The contents of the food vacuoles are rather difficult to identify. In some cases they appear filled with rod-like bodies which resemble bacteria; and in other vacuoles one can see objects which look very much like the nuclei from the intestinal mucosa cells of the host. No algæ or diatoms have been observed in any of the food vacuoles.

Other than food vacuoles, the endoplasm contains large quantities of fat stored as droplets. These are beautifully demonstrated with the use of Sudan III. The fat droplets are found most abundant in the region anterior to the food vacuoles and surrounding the macronucleus (Fig. 3, *A, ft. bo.*). Osmic acid is of value in the demonstration of these fat droplets; however, it preserves all of the other structures of a lipoidal nature and is, therefore, not so specific as Sudan III.

Another structural feature of specimens fixed in F. W. A. is the appearance in the anterior region of a series of vesicular structures (Fig. 3, *B, ves.*) which appear to empty, by means of slender ducts through the pellicle, to the exterior. These vesicles vary in size depending upon their distention. Two or more are often found united to the same duct. They appear to be associated more with the ventral than the dorsal surface. Their contents are of a homogeneous nature and from their reaction with osmic acid suggest a dilute lipoidal secretion. Although traces of them have been found in sections of individuals treated with fixatives other than F. W. A.; all attempts at their demonstration in the living ciliate have been unsuccessful.

The macronucleus is large, measuring on the average about 28 × 22μ and varying from ovoid to ellipsoid in shape with its long axis at right angles to the longitudinal axis of the animal. In sectioned material, the macronucleus shows a number of deeply-staining spherical bodies of various sizes embedded in its less dense matrix (Fig. 5, *C, mac.*). These deeply staining bodies fail in some cases to stain after the Feulgen reaction, in which case the matrix is lightly colored and contains areas showing the places occupied by the bodies which are deeply stained when treated with iron-hæmatoxylin.



4. Macronucleus of *E. borealis* in various stages of reorganization. *A* to *F,* macronucleus in one to seven parts respectively.

The micronucleus is always single, vesiculate (Fig. 5, *C, mic.*) and usually spherical, measuring about 5μ in diameter; and has always been observed to lie above the macronucleus, never imbedded in it.

A study of about 600 specimens fixed during the day gave but three individuals showing any signs of fission. Division was found to take place as described by Hentschel (1924). A number of individuals exhibited stages in a process of macronuclear reorganization in which the original macronucleus had divided into as many as seven parts, all arranged in an orderly fashion (Fig. 4, *A* to *F*). In all cases the micronucleus appeared in a normal resting stage. In some 5700 individuals examined, 5538 contained a normal resting macronucleus (Fig. 4, *A*); 103 contained 2 macronuclei (Fig. 4, *B*); 34 contained 3 macronuclei (Fig. 4, *C*); 21 contained 4 macronuclei (Fig. 4, *D*); 1 contained 6 macronuclei (Fig. 4, *E*); and 3 showed 7 macronuclei (Fig. 4, *F*). In every instance, the volume of the macronuclei in each individual, irrespective of number, seems to approximate that of a resting macronucleus.

Fibrillar System

Throughout sections of material fixed in Schaudinn's or Bouin's, there are found traces of a delicate and persistent system of intercommunicating fibers and fibrils. In reconstructing this system as a whole and in its present description, it has been considered as a neuromotor system. This system in *E. boreale* consists of three distinct groups of fibers and associated structures: (1) the stomatostyle with its dorsal and ventral anterior horns and their adoral fibers, the labial fibers, the pharyngeal fiber, and the circumpharyngeal rods; (2) the fibrillar center or motorium with its anterior, posterior, and marginal strands; and the posterior fibrillar center with its associated strands; and (3) the transverse commissural network integrating the cilia.

(1) Within the dorsal margin of the peristome, there is imbedded a curved bar, forked at its anterior end, for which the writer proposes the name *stomatostyle* (Fig. 5, *C, stom.*), because of its obvious nature as a support for the dorsal wall of the buccal cavity. The fork at the anterior end is composed of a dorsal horn which sends a strand of adoral fibers about the antero-dextral border and a ventral horn with fibers running to the basal granules of the oral cilia (Fig. 2, *B, bas. gra'*) as well as to the antero-ventral region. A branch also comes from the base of the ventral horn to become the main fiber of the pharyngeal area (Fig. 5, *C, phar. fib.*). From the manner in which the stomatostyle fragments when injured during sectioning, it appears to consist of a viscous semi-solid enclosed by a membrane. From its position and structure it seems to have a definite function as a supporting bar, or rod, for the frontal lobe which overhangs the buccal cavity. Its posterior end terminates near the pharynx (Fig. 5, *B*). Circumscribing the anterior end of the pharynx, there is a set of at least three heavy fibers or rods which are associated with both the labial membranes of the cytostome, and the posterior region of the stomatostyle (Fig. 5, *B* and *D*). Besides the principal pharyngeal fiber which extends posteriorly to lose itself in the region about the contractile vacuole, there are also

a number of accessory fibrils found in the anterior region of the pharynx (Fig. 5, *B, phar. fib.*). About the lips of the cytostome are found fibers which appear to have a connection with the ventral horn as well as the circumpharyngeal fibers.

Ventrad to the midregion of the stomatostyle is found (2) a fibrillar center or motorium (Fig. 5, *C, fib. cen.*); a clump of darkly staining granules which is a common junction for fibers and fibrils from all parts of the organism. From this center, delicate fibers pass in abundance to the anterior region, which, with its ciliary rootlets, presents a very complex appearance. Likewise, fibers are sent to the stomatostyle, as well as to the walls of the buccal cavity. Clearly defined strands of fibers run to both the right and left margins (Fig. 5 *C, ri.* and *le. mar. fib.*) as well as a large loose strand which passes posteriorly to the posterior fibrillar center (Fig. 5, *C, pos. fib. cen.*). The posterior fibrillar center in turn acts as a distributing point for fibers leading to all parts of the posterior region. The appearance of this structure (*pos. fib. cen.*) varies from individual to individual. In some specimens it is similar to that which has been figured; and in other cases it appears to be composed of a knot of interlocking fibers. The only reason, other than its morphological aspect, to suppose it to be a part of the neuromotor system is suggested by the behavior of this animal when in contact with unfavorable conditions. It has been repeatedly observed that whenever a specimen of *E. borealis* swims head-on into a toxic solution the anterior body region appears to be paralyzed and while in this condition the animal will back away from the unfavorable medium, wheel about, and move to a more suitable environment, solely through the use of the cilia of the posterior body region, suggesting that the posterior fibrillar center may be an auxiliary motor control center for the posterior body region.

The distal ends of most of the fibers described above terminate in fibrils which are closely associated with (3) the transverse commissural fibers found at the base of the ectoplasm and form a network interconnecting all of the basal granules (Fig. 5, *E* and *F*).

The fibers in general show a high degree of contractility as well as elasticity. One often finds in a single fiber regions of irregular diameter, suggesting a partial contraction of the fiber. There is no evidence at present to credit these fibers with functions as myonemes. All observations seem to point to their great elasticity as a device to accommodate the movement of a very flexible body as it progresses through a medium filled with the debris common to a sea urchin's intestinal tract. Thus, the posterior fiber is usually found in a very loose condition with its fiber greatly looped or loosely coiled as though it were capable of great flexibility to accommodate the body movements of the organism.

The neuromotor system, then, consists of three principal groups of structures: (1) the stomatostyle with its associated fibers connecting all parts of the cytostomal area and doubtless the controlling mechanism of the ingestatory apparatus; (2) the anterior and posterior fibrillar centers with their associated fibers leading to all parts of the cytostome and pellicle, of which, because of its greater complexity, the anterior fibrillar center seems more nearly to approach the nature of a coördinating center or motorium than any other structure in the body of the animal; and (3) the pellicular system of commissural fibers which connect the basal granules and coördinate the ciliary movements.

Discussion

One receives the impression, from a study of its internal morphology, that *E. borealis* is a rather highly specialized ciliate; an impression somewhat incongruous to that gained from observing the living animal. Perhaps the most striking feature of its morphology is the region about the cytostome as well as that structure itself. The external features of the mouth are finely constructed of ectoplasm which appears unspecialized and of such a nature as to presuppose feeding habits requiring food of a soft or liquid nature. That is to say, by structural components alone the nature of the food of this ciliate appears limited. When one examines the food vacuoles which so abundantly fill the posterior body region, one can find in them only material suggesting bacteria or bodies somewhat like the nuclei from the cells of the intestinal mucosa. Knowing that this animal has a habit of fastening itself ventrally to the surface of the intestinal mucosa, and trying further to give some function to the secretory vesicles of the anterior region; one is inclined to the consideration of this ciliate as being definitely parasitic and using its anterior vesicles as an agent in the secretion of cytolytic enzymes, thus enabling it to attack the intestinal mucosa of its host.

Closely associated with the cytostome are a number of fibers and rods, obviously of a supporting nature, *i.e.,* the stomatostyle, the fibers of the labial membranes, and the rods about the anterior pharyngeal region. These structures are usually found in a taut condition and seem to be of a more definitely supporting nature than do the fibers associated with the rest of the endoplasm. These latter are very delicate, orderly, and are usually found in a loosely coiled or attenuated condition suggesting a function as conducting fibers or neuroids. These neuroidal fibers radiate from two interconnecting centers, the more complex being in the anterior region while the simpler is found in the posterior region, above the contractile vacuole. It must be borne in mind that these interpretations of the writer are based solely on the morphological evidence and remain to be proved through actual experimental studies.

Many of the European workers are of the opinion that the transverse commissural fibers alone are sufficient for the control of the body movements and locomotion among the ciliates (Reichenow, 1927); and that any other fibrillar structures are of a supporting nature. On the other hand, most of the workers in America feel that the internal fibrillar system is an integral structure associated with all neuromotor activities. The only experimental evidence with which the writer is familiar, concerning either view, and confirming the latter, is based upon the observations of Taylor (1920), who was able, by means of micro-dissection methods, to present definite experimental data on the function of the neuromotor apparatus in *Euplotes*. As yet, one (Ten Kate, 1927) does not seem justified in making generalizations to cover all of the observations now on record for the fibrillar systems of ciliates. The diversity of the structures described leads one to the feeling that perhaps more than one system of structures has been described in some cases, and that in others, there may be some important features which, due to the technical difficulties involved, have been overlooked. It would seem that the workers on the Continent have been drawing their conclusions from something quite different from that used as a basis of thinking in America.

The view that the fibrillar system is merely structural is based principally on the observations made upon various members of the genus *Balantidium* (Bezzenberger, 1904; Ten Kate, 1927; and Ray, 1932). This is a notable piece of work for it gives us a rather complete picture of the close relationship among the various species of this genus as portrayed by their fibrillar structures, which in many cases are homologous. The interpretation of the fibers in the anterior region of *Balantidium* as being of a supporting or structural nature seems entirely logical. However, this generalization concerning the fibrillar system of *Balantidium* cannot well be applied to all other ciliates, some of which have been shown to be so vastly different morphologically.

It is not inconceivable that a structure such as the stomatostyle of *E. borealis* may possess all three properties ascribed for various fibrillar elements; besides being supportive, it may also be contractile as well as conductile; and likewise, other fibrillar elements may have more than one function. In considering these structures under discussion, one must keep ever in mind the difficulties involved in making observations on these delicate organelles, for their demonstration is dependent upon carefully controlled technique in both fixation and staining. It would seem that, provided the technique for micro-dissection could be sufficiently refined, *E. borealis* would offer exceptional material for the study of the fibrillar system. This species is obtainable in great abundance at any time of the year and will live in cool sea water of the proper concentration for several weeks.

SUMMARY

1. *Entodiscus borealis* (Hentschel), a ciliate infesting the alimentary tract of *Strongylocentrotus dröbachiensis,* when studied intensively, reveals a number of interesting manners of behavior as well as a series of complicated structures in connection with the cytostome and the fibrillar system.

2. The buccal cavity, guarded by a pair of membranous lips, has hanging from its roof a group of very long cilia. Embedded in the margin along its right wall is found a supporting rod for which the name *stomatostyle* is proposed.

3. Coördinating this stomatostyle as well as other parts of the body are two intricate sets of fibers arranged about two interconnecting centers: the *anterior fibrillar center,* and the *posterior fibrillar center.* These fibrillar structures, along with the transverse commissural fibers, found just beneath the pellicle, are interpreted as a well-developed neuromotor system.

4. After fixation with F. W. A. numerous vesicles, with outlets through the pellicle, are demonstrated in the anterior region. In considering the contents of the food vacuoles, these secretory vesicles suggest themselves as possible agents in producing cytolytic enzymes through whose aid the ciliate is able to attack and ingest portions of the intestinal mucosa of its host. In various regions about the cytostome and the pharynx there are found groups of secretory (?) granules.

5. *E. borealis* will exist for as long as 3 weeks in hanging drop cultures in sea water. Reproductive activity seems to occur only in the body of its host. The infestation appears to be maintained through early oral infection of young sea urchins.

REFERENCES CITED

Bezzenberger, E., 1904. Über Infusorien aus asiatischen Anuren. *Arch. f. Protist.,* **3:** 138.

Hentschel, C. C., 1924. On a New Ciliate, Cryptochilum boreale nov. sp., from the Intestine of Echinus esculentus Linn., together with some notes on the Ciliates of Echinoids. *Parasitol.,* **16:** 321.

Lynch, J. E., 1929. Studies on the Ciliates from the Intestine of Strongylocentrotus. I. Entorhipidium, gen. nov. *Univ. Calif. Publ. Zoöl.,* **33:** 27.

Madsen, von Holger, 1931. Bemerkungen über einige entozoische und freilebende marine Infusorien der Gattungen Uronema, Cyclidium, Cristigera, Aspidisca und Entodiscus gen. nov. *Zool. Anz.*, **96**: 99.

Ray, Harendranath, 1932. On the Morphology of Balantidium sushilii n. sp., from Rana tigrina Daud. *Jour. Roy. Mic. Soc.*, **52**: 374.

Reichenow, Edward, 1927–29. Doflein's "Lehrbuch der Protozoenkunde" (ed. 5), p. viii–1262. Jena.

Taylor, C. V., 1920. Demonstration of the Function of the Neuromotor Apparatus in Euplotes by the Method of Microdissection. *Univ. Calif. Publ. Zoöl.*, **19**: 403.

Ten Kate, C. G. B., 1927. Über das Fibrillensystem der Ciliaten. *Arch. f. Protist.*, **57**: 362.

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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

II. BACTERIA CONCERNED IN THE CYCLE OF NITROGEN IN THE SEA [1]

SELMAN A. WAKSMAN, MARGARET HOTCHKISS,[2] AND
CORNELIA L. CAREY [3]

(From the Woods Hole Oceanographic Institution and New Jersey Agricultural Experiment Station)

INTRODUCTORY

Among the various elements which are essential for the growth of plant and animal life in the sea and which are present in minimum concentrations, nitrogen occupies a prominent place. Neither the plant nor the animal forms are able to use the gaseous nitrogen of the atmosphere, a function now known to be limited chiefly to certain specific groups of bacteria, and depend for their growth upon the combined nitrogen present in the sea; but even this form of nitrogen must be in a soluble, mineralized state before it can be assimilated by the plants. The plants transform the soluble forms of nitrogen into complex organic forms; upon the death of the plants, as well as of the animals which feed upon them, the nitrogen is brought again into circulation, through the action of a number of bacteria, in the form of ammonia. However, before this nitrogen is again assimilated by the marine plants, the ammonia is usually oxidized first to nitrite and then to nitrate by the action of certain other bacteria. The nitrate, if not consumed by the plants especially in the surface layers of the sea, may be reduced, under certain conditions, by the action of still other bacteria to nitrite, to ammonia, and to gaseous forms of nitrogen. Some bacteria may compete in the sea with higher plants for the available inorganic nitrogen, in the presence of certain carbohydrates, and thereby transform this nitrogen into organic compounds.

The bacteria are thus concerned in the sea with at least five distinct

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[1] Contribution No. 18 of the Woods Hole Oceanographic Institution and Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Soil Microbiology.

[2] Department of Bacteriology of the New York Homeopathic Medical College.

[3] Department of Botany, Barnard College, Columbia University.

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processes which are based upon the circulation of nitrogen, and which affect in numerous ways, directly and indirectly, the growth of plant and animal life in the sea, namely (1) liberation of nitrogen as ammonia, (2) oxidation of ammonia to nitrite and nitrate, (3) reduction of nitrate to nitrite and atmospheric nitrogen, (4) fixation of nitrogen, and (5) assimilation of inorganic forms of nitrogen. The first and last of these processes have been discussed at some length in a previous publication (48). The investigations reported in this paper are concerned with the other three processes of nitrogen transformation.

OCCURRENCE OF NITRIFYING BACTERIA IN THE SEA

Historical

The formation of nitrate in the sea, as a result of oxidation of ammonia, is usually considered as the final step in the transformation of nitrogen, before it is made again available for assimilation by green plants; this does not exclude, of course, the probability that ammonia itself, the final nitrogenous product of decomposition of organic matter by bacteria, and nitrite, the first product of oxidation of ammonia, can also be utilized by marine algæ and by the chlorophyl-containing members of the plankton, as sources of nitrogen. Among the various marine problems, for which bacteria are believed to be responsible, none has aroused more discussion and greater interest than the process of nitrate formation in the sea, with the possible exception of nitrate-reduction by bacteria. This interest is due to the importance of nitrate formation in the metabolism of the sea and to the difficulty of studying the bacterial agents responsible for this process. An exact analogy is found in the study of the agents of nitrification on land: even forty years after the respective organisms have been isolated and cultivated by Winogradsky, papers still continue to appear which not only question the rôle of these organisms in the process but frequently doubt their very existence.

Boussingault looked upon the ocean, in 1860, as an immense reservoir of nitrogen in a combined form. Schlösing (43) demonstrated, in 1875, that while land waters are richer in nitrate, sea waters are richer in ammonia. It was recognized, however, that both ammonia and nitrate result from the decomposition of nitrogenous organic matter in the sea. Natterer (36) measured the NH_3, NO_2, and NO_3 content of sea water and found that the first was most abundant, while the last two were present in mere traces or were entirely absent; he believed that nitric acid produced by electric discharges sooner or later reaches the sea, enters there into organic combination, and is finally transformed to ammonia; this diffuses then into the atmosphere, and contributes again to the growth of plants on land; these results were not confirmed, however, by subsequent investigators. Brandt (9, 10) stated emphatically that the cycle of nitrogen in the sea is essentially not very different from that on land.

The first suggestion concerning the existence in the sea (Gulf of Naples) of bacteria responsible for the process of nitrification was made in 1898 by Vernon (45). Baur and Brandt (2) are believed to have demonstrated in 1900 the presence of nitrifying bacteria in the sea: two out of three mud samples inoculated into a solution containing ammonium salts gave active nitrification; however, sea water itself seemed to be free from the organisms concerned in this process. These results could not be confirmed by Gran (19) and Nathanson (37), who were unable to demonstrate these organisms either in the Norwegian fjords or in the Gulf of Naples. Gran found nitrifying bacteria only close to the shore. This led Nathanson to conclude that when bacteria are found in the sea not far from land, it is due to their introduction from the land soils by streams and land drainage. Gran and Nathanson adhered, therefore, to the earlier hypothesis of Schlösing that nitrates are not formed in the open sea, but are brought there from the outside, either from the atmosphere or from land.

Brandt argued that, if the nitrate comes into the sea either from the atmosphere or from land by means of streams and rivers, one would expect to find nitrate more abundant in the surface layers of the sea rather than in the lower depths, which is contrary to actual facts. The abundance of nitrate in the deeper layers of water led Brandt to conclude that nitrification takes place chiefly in the sea bottom or close to it. It was recognized, however, that close to the mouth of the rivers the relative concentration of nitrate was greater than in the open sea, but this was also found to hold true for other forms of nitrogen, namely ammonia and protein.

Thomsen (44) demonstrated the presence of nitrite-forming bacteria in considerable abundance in the sea bottom, although they were absent in the sea water and on algal material; the nitrate-forming organisms were also present in the bottom material, but only close to shore. The organisms responsible for the two processes, namely *Nitrosomonas* and *Nitrobacter,* were isolated from the sea and were found to be morphologically the same as the corresponding forms isolated by Winogradsky from land soil. The marine nitrite bacteria were considered as adaptation forms, their optimum temperature being similar to that of the bacteria from land. Thomsen found nitrifying bacteria not only in the mud from the Gulf of Naples, but also from the Kiel Bay and the North Sea. He believed that the negative results of

Nathanson were due to the nature of the medium which he had used for demonstrating the presence of these organisms, and to the fact that the cultures were not incubated sufficiently long and that the temperature was unfavorable. The occurrence of these bacteria on the bottom of the sea and not in the free water itself was explained by the fact that the water contains only traces of ammonia, while the continuous decomposition of plant and animal residues in or on the bottom supplies the necessary substrate for their action.

Issatchenko (24, 25) reported in 1908 that he found nitrifying bacteria in the water of the northern Arctic Sea; these organisms were present in the bottom material of the Catherine Coast (Murmansk) and of the North Ice Sea, as well as of the high seas; they were absent, however, in the surface water. Only nitrite-forming bacteria were found in the sea bottom, but not the nitrate-formers. In a later contribution (26), Issatchenko has shown that the nature of the sea bottom material is of importance in determining the abundance of nitrifying bacteria; these organisms were more abundant and could be more readily demonstrated in sandy bottoms and in shell-rich bottoms than in clay bottoms.

Liebert (30) attempted, in 1915, to isolate nitrifying bacteria from the water of high seas and from the ocean bottom; his results were entirely negative. He concluded that sea waters at a distance from land contain no bacteria capable of oxidizing ammonia and nitrate, due to the low content of these nitrogenous compounds in the sea. Marine mud from the North Sea also gave negative results, except in close proximity to shore. However, both nitrite and nitrate formation took place in the Zuyder Sea. Liebert believed that the FeS present so abundantly in the bottom of the ocean may play an important rôle in the oxidation of the ammonia in marine bottoms. Berkeley (14), in 1919, tested sea water for the presence of nitrifying bacteria with negative results, even after three months incubation of the cultures. As a result of a series of investigations, Lipman (31) concluded that although nitrifying bacteria are absent in the water of the open sea, they are present in the sea bottom, such as calcareous sand. Similar results were reported by Harvey (21–23).

On the basis of these results, Brandt (11) concluded in 1926, with much justification, that the results so far obtained are sufficient to establish definitely the fact that bacteria capable of oxidizing ammonium salts are completely lacking in surface waters, but are present in marine bottoms.

The possibility of photo-chemical oxidation in the sea of ammonia to nitrite and even to nitrate has recently been suggested (51). It has been known (39) that solutions of ammonia and ammonium salts exposed to sunlight, in the presence of small quantities of a photo-sensitizer, will give rise to nitrite, especially in alkaline solutions. TiO_2, ZnO, CdO, and others act as photo-sensitizers. The photochemical formation of nitrite and nitrate can, at best, however, explain only partly the origin of nitrates in the sea, since it must take place in the surface layers, with the formation of ammonia preceding this process. The fact that the nitrate is largely found in the lower layers of water would tend to emphasize the probable limitation of this process in the formation of nitrate in the sea.

Experimental

In an attempt to study the occurrence and activities of nitrifying bacteria in the sea, it was deemed essential to establish at first the conditions favorable for the growth of these organisms. One deals here with bacteria highly selective in their metabolism, very sensitive to environmental conditions, and specific in their food requirements, as was amply shown for the corresponding organisms universally active and abundant in land soils. Whether or not the bacteria responsible for nitrite and nitrate formation in the sea are as highly sensitive to conditions as the land bacteria, the first prerequisite in such investigations was to select a medium favorable for their development under artificial laboratory conditions. Several preliminary experiments were, therefore, carried out in order to test sea water as a medium and the most optimum conditions for the growth of these organisms.

At first, fresh sea water, to which 0.05 per cent K_2HPO_4, some $CaCO_3$, and varying amounts of ammonium salts had been added, was used. These experiments have shown that neither nitrate nor nitrite was formed in such a medium. However, when fresh sand or mud was introduced into the flasks, active nitrite formation took place. The results of a typical experiment are reported in Table I. The medium used in this experiment consisted of,

Fresh sea water	1000 cc.
K_2HPO_4	1 gram
$(NH_4)_2SO_4$	1 gram
$CaCO_3$	5–10 grams

This medium was placed in a series of flasks; these were plugged with cotton and the medium left unsterilized. Some of the flasks received 30-gram portions of fresh bottom mud taken off the shore of Gay Head, while others did not receive any mud. At frequent intervals nitrite tests were made by removing 1-cc. portions of the culture, by the use of sterile pipettes. The test was made with a mixture of sulphanilic acid and diethyl β-naphthylamine solutions. One cc. of culture was diluted with 5 cc. of distilled water and 1-cc. portions of each of the two reagents added. The color was read after 5 or 10 minutes.

The results brought out in Table I clearly demonstrate the fact that sea water either does not contain any bacteria capable of oxidizing ammonium salts to nitrite or is not a favorable medium for the development of these bacteria. In the culture to which marine mud has been added active nitrite formation took place, even after 20 days incubation; the amount of nitrite formed increased rapidly on further incubation. This can be due either to the presence of nitrifying bacteria in the mud or to the fact that the mud made the sea water medium more favorable for the development of these organisms.

TABLE I

Nitrite formation in sea water medium and in sea water medium to which fresh marine mud has been added.

Tr = trace of nitrite; 0 indicates negative test; + = positive nitrite test; +++ = extensive nitrite formation; ++++ = maximum nitrite reaction; — indicates culture discarded.

Treatment of culture	Days of incubation of cultures						
	6	15	20	24	28	33	38
Water alone	0	0	0	0	tr	tr	tr
Water alone	0	0	0	0	tr	—	—
Water and mud	0	0	+	+++	++++	++++	++++
Water and mud	0	0	+	+++	++++	—	—

Another important point to be noted from this experiment is that only the tests for nitrite were positive, while the tests for nitrate were all negative, even in the mud cultures. Attention has been called, in the review of the literature, to the comparative rarity in the sea of the nitrate-forming bacteria, as compared with the nitrite-formers. This would seem to be an anomaly, since there is very little nitrite present in the sea as compared with nitrate. If one remembers, however, the fact that the nitrate bacteria are highly sensitive to traces of free ammonia, which would be produced from the ammonium salt in an alkaline medium, one would expect to find these organisms, if they are present at all, only after all the ammonia has been oxidized to nitrite. The tests were continued for a longer period, but no trace of nitrate could be detected within 57 days of incubation; however, after 60 to 62 days, the mud containing cultures gave a definite test for nitrate, and after 77 to 84 days, the test became strongly positive.

An experiment was then started to determine the influence of the initial concentration of ammonium salt added to the original medium upon the development of nitrite and nitrate-forming bacteria, since some of the previous investigators (31, 33) considered this to be an important factor. This experiment was carried out as follows: Twelve 250-cc. Erlenmeyer flasks received 40-gram portions of washed sea sand and 2 grams $CaCO_3$; the flasks were plugged with cotton and sterilized for 1 hour under pressure. Sixty-cc. portions of sea water containing 0.1 per cent K_2HPO_4, previously heated for 1 hour at 70° C., were then added to the flasks, as well as varying amounts of an ammonium sulfate solution, also heated previously at 70° C. Five of the flasks were inoculated with fresh surface water taken from Great Harbor, near the Oceanographic Institution wharf, five flasks were inoculated with sea bottom mud from off the shore of Gay Head and 2 flasks with 4 drops of an enriched culture of nitrite-forming bacteria grown in a sand-sea water medium.

TABLE II

Effect of varying concentrations of ammonium sulfate upon nitrite formation in sea water-sand medium.

0 indicates negative test; + = positive nitrite test; ++ and +++ = extensive nitrite formation; ++++ = maximum nitrite formation.

Concentration of $(NH_4)_2SO_4$ in 60 cc. of medium	Sea water inoculum					Mud inoculum					Enriched culture inoculum			
	Days of incubation					Days of incubation					Days of incubation			
	3	7	12	17	20	3	7	12	17	20	3	7	12	17
mgm.														
0	0	0	0	0	0	0	0	+	++++	++++				
10	0	0	0	0	0	0	0	++	++++	++++				
25	0	0	0	+	+++	0	0	0	+	++	0	0	+	++++
50	0	0	0	+	++	0	0	+	+++	++++	0	0	+	++++
100	0	0	0	0	0	0	0	0	++	++++				

The concentration of the ammonium salt and the nature of the inoculum were found to have an important effect upon the rapidity of nitrite formation, as shown in Table II. The use of sea water as an inoculum gave no nitrite formation with either the highest or the lowest concentrations of the ammonium salt; however, a positive reaction was obtained in the cultures containing 25 and 50 mgm. of the salt, after 17 days incubation. The cultures inoculated with mud gave a positive nitrite test in some cultures in 12 days and in all cultures in 17 days. The mud cultures behaved in a manner similar to the enriched culture of the nitrite-forming organism.

Some of the cultures in the above experiment were incubated for a longer period than that reported in Table II. The culture containing 10 milligrams of ammonium sulfate and inoculated with mud gave very abundant nitrite formation up to 34 days; after 42 days, however, the nitrite disappeared. It had been completely and rapidly converted to nitrate.

In the previous experiments, both the water and the marine bottom material were obtained in the proximity of land. The following experiments deal with the occurrence of nitrifying bacteria in the Gulf of Maine and on George's Bank, at a considerable distance from shore. Material for this experiment was obtained on a cruise of the " Atlantis," which took place during August 1–5, 1932, and which has been described in detail elsewhere (50). For this purpose a series of flasks were prepared containing the following materials:

Table III

Presence of nitrite-forming bacteria in the sea water of the Gulf of Maine (Stations 1329, 1330, 1331, and 1332) and Georges Bank (Stations 1333 and 1334) at different depths.

Tr = trace; 0 indicates negative test; + indicates positive reaction; ++ = extensive nitrite formation; +? indicates doubtful reaction. For map showing location of stations, see Rakestraw, 1933, *Biol. Bull.*, **64**: 150.

Station No.	Depth of water	Days of incubation			
		10	15	20	23
	meters				
1329	Surface water	0	+?	0	0
1329	Above bottom	0	0	0	0
1330	Above bottom	0	0	0	0
1331	Surface water	0	0	0	0
1331	30	tr	tr	tr	+
1331	50	tr	+	+	+
1331	100	tr	0	0	+
1331	215	tr	+	+	+
1331	Above bottom	0	0	0	0
1332	Surface water	0	+	+	++
1332	50	0	+	+	+
1332	100	0	+	+	+
1333	Surface water	0	tr	tr	tr
1334	Surface water	0	tr	0	tr
1334	Deep water	0	tr	tr	tr

Sand	15	grams
$CaCO_3$	1	gram
K_2HPO_4	0.005	gram
$(NH_4)_2SO_4$	0.01	gram

The flasks were plugged with cotton and sterilized, at 15 lbs. pressure, for 15 minutes. A standard solution of ammonium sulfate was sterilized separately and added to the sterile flasks. At the various stations visited about 50–60-cc. portions of fresh sea water, brought up from the different depths, by means of sterile glass containers, were added to the flasks immediately after the samples were obtained. To determine the occurrence of nitrifying bacteria in the marine bottom of the open sea, a medium similar to the above and containing 75–cc. portions of sea water with only 5 milligrams of $(NH_4)_2SO_4$ per flask was prepared. This medium was sterilized for 1 hour in flowing steam (100° C.). The flasks were inoculated, after returning from the cruise, with small quantities (about 1 gram) of fresh mud, obtained in the Gulf of Maine under sterile conditions; this was done by removing carefully the inner part of a core of mud.

Table IV

Occurrence of nitrite-forming bacteria in the marine bottom of the Gulf of Maine (Stations 1329 and 1330) and Georges Bank (Station 1336).

Tr = trace of nitrite; + = positive nitrite test; ++ and +++ = extensive nitrite formation. For map showing location of stations see Rakestraw, 1933, *Biol. Bull.*, **64**: 150.

Station No.	Depth of mud	Days of incubation			
		2	6	11	15
	cm.				
1329	0–30	+	++	+++	+++
1329	30–60	0	++	++	+++
1329	60–90	0	tr	tr	tr
1330	0–30	+	+++	++	+++
1330	30–60	0	tr	tr	tr
1330	60–90	tr	tr	tr	tr
1336	Surface layer of sand bottom	0	+	+	++
Control	0	0	0	0	

The results of these experiments on the presence of nitrite-forming bacteria in the sea water and in the marine bottom of high seas, are reported in Tables III and IV. These results show that the free water in the high seas is either entirely free from nitrifying bacteria or contains only very few cells of these organisms. In the case of the marine bottoms, however, positive nitrite formation was obtained in the case of the mud bottoms (Stations 1329 and 1330). Even within two days incubation these bacteria seem to be present in the mud to quite considerable depths. The sand bottom also gave active nitrite formation, somewhat more slowly than the mud bottoms.

In addition to the cultures containing ammonium salts, other cultures containing nitrites were prepared, but all attempts to obtain nitrate-forming bacteria in these media failed. However, when old cultures of nitrite-forming bacteria, in which nitrate-formation began to take place, were transferred to fresh media containing 5 mgm. KNO_2 in 30 cc. of medium, active nitrate formation took place within 7–8 days.

The following medium was finally adopted for the purpose of demonstrating the presence of nitrifying bacteria, using large test tubes instead of flasks, due to the ease of handling. The tubes contained 30 cc. sea water, 10 gm. washed sand, 1 gm. $CaCO_3$, 5 mgm. $(NH_4)_2SO_4$, and 5 mgm. K_2HPO_4. The medium was sterilized in flowing steam; the ammonium salt was sterilized separately, then added in aliquot portions, using sterile pipettes.

The nitrite-forming organism could be readily cultivated on the above medium; on repeated transfer, active cultures were obtained. By inoculating a silica-gel medium (thoroughly dialyzed in tap water and soaked in sterile sea water), to which an ammonium salt and calcium carbonate had been added, with an active liquid culture, abundant formation of nitrite on the plate took place within 14 days. This should facilitate greatly the isolation of the organism in pure culture, although for the purpose of the above experiments this was not considered essential.

The results presented here and other data of a similar nature are quite sufficient to demonstrate definitely that free sea water, especially at the surface of the sea, has either no nitrifying bacteria at all or only very few of these organisms. On the other hand, the sea bottom, mud or sand, has an active population of nitrifying organisms. The formation and accumulation of nitrate in the sea is probably due largely to the activities of these organisms. The processes of nitrite and nitrate formation take place in the sea bottom; the nitrate then diffuses into the water. The fact that it is much easier to demonstrate in culture the formation of nitrite than that of nitrate is due largely to the specificity of the organisms and conditions of cultivation.

REDUCTION OF NITRATES IN THE SEA BY BACTERIA

Historical

Nitrate is consumed in the sea not only by the green plants but also by bacteria, which either transform it into bacterial cell substance or reduce it to nitrite, ammonia, or atmospheric nitrogen. The activities of the last group of bacteria have aroused the interest of oceanographers. The increasing concentration of nitrate with the depth of the water in the open sea, the high nitrate content in temperate waters and its comparative poverty in tropical waters, the fact that nitrate is the final product of the transformation of nitrogen by bacteria, and further the fact that the phytoplankton and marine algæ seem to use this form of nitrogen in preference to any other, have all served to center the attention of marine investigators upon the transformation of nitrate in the sea. An attempt has actually been made to correlate not only the relative abundance of nitrate in the sea but also the abundance of plant and animal life with the activities of the nitrate-destroying bacteria.

If one is to admit the assumption that bacteria control largely the cycle of nitrate in the sea, especially its disappearance under certain conditions and not under others, one would have difficulty in explaining the common observation that nitrate and phosphate occur in the sea in a parallel manner. If one were to conclude that the nitrate balance in the sea is controlled by the activities of nitrate-forming and nitrate-reducing bacteria, one must also postulate that the formation and disappearance of phosphate in the sea are also controlled by the activities of comparable groups of bacteria. This may be true for the liberation of nitrate and phosphate, since nitrate formation is a resultant of a chain of processes brought about by a series of bacteria, while phosphate is liberated directly, in the decomposition process of plant and animal residues in the sea. On the other hand, although nitrate-reduction is known to take place under favorable environmental conditions, which include the presence of nitrate, an available source of energy, and the necessary bacteria, the reduction of phosphate in the sea, aside from its direct consumption by the phytoplankton, is still a matter of conjecture.

It is further essential to keep in mind the fact that the nitrate-reducing bacteria do not comprise a single group of organisms but several groups with distinct physiological properties, namely, (*a*) bacteria which reduce nitrate to nitrite only, (*b*) bacteria which reduce nitrate and nitrite to ammonia, and (*c*) bacteria which reduce nitrate and nitrite to atmospheric nitrogen and to gaseous oxides of nitrogen. Only the last group should be considered as true denitrifying bacteria; their activities may be of significance in the disappearance of nitrate in the sea. The products formed by the first two groups of nitrate-reducing bacteria can either be assimilated directly by the marine algæ and the phytoplankton or they can be oxidized back to nitrate by specific bacteria.

According to Audoynaud (1), both ammonium salts and nitrates are assimilated by the numerous small organisms inhabiting the sea, their bodies later sinking to the bottom and forming layers of various degrees of thickness. He, as well as Schlösing (43), had only a very vague idea concerning the return of the elements, especially of the nitrogen, to the atmosphere or to the sea, as a result of the decomposition of the organic residues.

The presence in the sea of bacteria capable of reducing nitrate, largely to nitrite, was demonstrated by Beijerinck (5) in 1890, by Fischer (15) in 1894, for phosphorescent bacteria, by Russell (42) in 1893, for the sea in the vicinity of Woods Hole, and by Vernon (45) in 1898, for the waters in the Gulf of Naples.

A decided stimulus to further investigations of nitrate-reducing bacteria was given by Brandt (9), in 1899, by his now famous hypothesis concerning the function of denitrifying bacteria in controlling the supply of available nitrogen in the ocean and, therefore, of plankton development. Brandt believed that most of the nitrogen compounds are brought into the sea from land by the rivers, streams, and drainage waters; without the activities of the denitrifying bacteria, nitrate concentration in the sea would soon reach a point where it would become injurious to living organisms. The more abundant plankton life in shallow waters, as compared with deep waters, was ascribed to the fact that, in the former, the available inorganic nutrients are distributed through a smaller volume of water, while in the deeper seas, the nutrients are distributed in much greater volumes of water. Plankton-rich seas were found to contain much more nitrate (and nitrite) than plankton-poor seas; a direct relation was thus shown to exist between the mass of plankton development and the amount of nitrate present. The conclusion was, therefore, reached that the abundance of nitrate controls the abundance of plankton. The tropical and sub-tropical seas are known to be comparatively poor in plankton, while the arctic seas are rich in plankton, especially in the summer; this phenomenon is just the opposite of that found in the case of land vegetation. This difference in the plankton development in the sea was explained by the difference in the concentration of available nitrogen compounds; the tropical climate favors active denitrification which results in the reduction of nitrate and the limitation of this most important plant nutrient in the sea.

These ideas resulted in an extensive study of the occurrence of denitrifying bacteria in the sea and their importance in marine processes. In 1902, Baur (2) reported the isolation of a denitrifying bacterium from the mud of an aquarium rich in nitrate; another form was isolated from the mud in the bay of Kiel. Gran (18) isolated from the sea, in 1901, several bacteria capable of reducing nitrate. He found that only at close proximity to shore, where organic residues are present in abundance and where large quantities of nitrate and nitrite are brought in from shore, is there any possibility that true denitrifying bacteria play an important part in the destruction of nitrate. Gran concluded that Brandt's hypothesis is untenable.

Feitel (14) has shown in 1903 that denitrifying bacteria are present in great abundance in the sea, at all depths, but more often on the sea bottom or in the water near it, where organic matter is abundant. These bacteria were found not only close to land but also at a considerable distance from shore; when isolated in pure culture, they were capable of bringing about active denitrification, especially at low temperatures. Nathanson (37) demonstrated in 1906 the presence of denitrifying bacteria in the Gulf of Naples. In 1908, Kühl (29) found that marine mud brings about active denitrification. In 1909, Gräf (17) isolated from the waters of the Atlantic and Indian oceans 17 forms of denitrifying bacteria; this common occurrence of nitrate-reducing bacteria, taken together with the observations of the increase in nitrate concentration with an increase in the depth of water and the decrease of nitrate concentration with an increase in temperature, was believed to substantiate Brandt's hypothesis.

Issatchenko (25) found denitrifying bacteria in the North Polar Sea, capable of growing at very low temperatures (1–3° C.). He concluded that the bacteria reducing nitrate to nitrite or to ammonia are widely distributed in the sea at various depths and are represented there by numerous types, which are normal inhabitants of the sea. The true denitrifying bacteria, which are also widely distributed in the northern seas, were considered of doubtful importance in bringing about any appreciable losses of nitrogen in the sea. The fact that these bacteria are able to develop and reduce nitrate at low temperatures (12) and the fact that warm seas are also rich in nitrates (16) led Issatchenko to deny the validity of Brandt's hypothesis.

According to Gran (20), appreciable denitrification may take place only in enclosed bays and fjords, where large amounts of organic matter are present, considerable nitrate is brought in from land, and, due to limited circulation, the amount of oxygen is limited.

This brief summary of the literature bearing upon Brandt's hypothesis, concerning the importance of denitrifying bacteria in the cycle of life in the sea, points to the fact that the weight of the evidence bears out the conclusions of Gran and Issatchenko, namely, that admitting the existence of denitrifying bacteria in the sea, their rôle in marine processes is far from being established; certainly their part in controlling the nitrogen content of the sea and, therefore, of plankton development is shown to be of minor importance.

Another hypothesis based upon the activities of denitrifying bacteria served to focus the attention of oceanographers on the rôle of these organisms in marine processes. Drew (13) attempted to correlate the activities of these bacteria with the process of calcium carbonate precipitation in the sea: nitrate-reducing bacteria decompose calcium salts of organic acids present in the sea; this results in the precipitation of the calcium as calcium carbonate. However, further studies on the occurrence of denitrifying bacteria in tropical seas and on the mechanism of calcium carbonate precipitation did not tend to confirm this hypothesis. Lipman (32) found that precipitation of $CaCO_3$ will take place in a suitable medium even in the absence of nitrate. Whatever the final explanation of the mechanism of calcium precipitation in the sea (32, 34, 3, 4), one is justified in concluding that the original hypothesis of Drew has not been substantiated by subsequent investigators. In order to bring about such extensive precipitation, large quantities of nitrate would have to be formed and still larger quantities of organic acids produced in the sea, two processes which have not been sufficiently established.

However, even if these two hypotheses concerning the importance of denitrifying bacteria in marine processes may be considered, for the present at least, as untenable, the fact remains that the sea harbors many bacteria capable of reducing nitrate to nitrite, to ammonia, and to atmospheric nitrogen (38, 35). Only a detailed study of the occurrence of these organisms in the sea and of their biochemical activities may enable one to interpret their true function in marine processes.

Experimental

Most of the media used by previous investigators for the growth of nitrate-reducing bacteria contained peptone (3, 34), which could serve both as a source of energy and of nitrogen. However, the use of an organic nitrogen compound for the growth of these bacteria tends to confuse the results obtained, since some of the products, such as ammonia, may either be due to the reduction of the nitrate or to the decomposition of the protein. In the following experiments, a synthetic medium was employed, with nitrate as the only source of nitrogen and with various carbohydrates or salts of organic acids as sources of energy.

In the first experiment, a study has been made of the relative abundance of nitrate-reducing bacteria in sea water and in marine mud. For this purpose the following medium was found to give good results:

Sea water	1000	cc.
Ca-acetate	10	grams
$NaNO_3$	1.0	gram
K_2HPO_4	0.5	gram
$FeSO_4 \cdot 7H_2O$	0.01	gram

Thirty-five-cc. portions of this medium and 10-gram portions of sand were placed in a series of large test tubes; these were plugged with cotton and sterilized, by heating at 70° C. for 4 hours. By the use of this medium and the dilution method, denitrifying bacteria, or organisms capable of reducing nitrate to gaseous forms of nitrogen, were found in dilutions of 1 cc. and 0.1 cc. of sea water, taken near shore, and in 0.0001 gram of mud.

The above method of sterilization proved to be unsatisfactory, since some of the controls contained bacterial spores. A study has, therefore, been made of the influence of sterilization of the medium upon the growth of nitrate-reducing bacteria. The results of this experiment are presented in Table V. Autoclaving the medium did not render it unfit for the development of these bacteria, hence one could feel fully justified in proceeding with this method of sterilization.

The above medium was used in the investigation of the distribution of nitrate-reducing bacteria in sea water and in mud in the Gulf of Maine. The results of this experiment, reported elsewhere (50), brought out the fact that bacteria reducing nitrate to nitrite are universally distributed in the sea. However, the occurrence in the open sea of bacteria reducing nitrate to atmospheric nitrogen is very limited. Only in the case of one station, with 0.1 gram of mud as the inoculum, was positive denitrification observed, when the nitrate was rapidly reduced to gaseous nitrogen. In none of the other seven stations studied and in none of the water samples obtained from the various depths could the presence of these organisms be demonstrated. The bacteria reducing nitrate to nitrite were found to be much more abundant in the mud than in the free water. Most of the nitrate-reducing organisms present in the water belonged to the non-spore-forming bacteria; how-

TABLE V

Influence of sterilization of medium upon the development of denitrifying bacteria. Marine mud used for inoculation and cultures incubated for 3 days.

Dilution of mud	70° C., 2 hours*	100° C., 1 hour	15 lbs. pressure, 15 minutes	Filtration of culture through Seitz filter*
0.01	Gas	Gas	Gas	Gas
0.001	Gas	Gas	Gas	0
0.0001	Pellicle, no gas	Pellicle and gas	Gas	0
0.00001	0	Pellicle, no gas	Medium turbid, surface ring	0
Control	0	0	0	0

* Tubes and sand sterilized under pressure.

ever, several tubes inoculated with various dilutions of mud showed unmistakably the presence of spore-forming organisms. The actual amount of nitrate reduced in all the cultures reducing nitrate to nitrite was comparatively small.

In order to study the process of reduction of nitrate under controlled laboratory conditions, especially for the purpose of determining the influence of the energy source upon the reduction of nitrate to nitrite and to atmospheric nitrogen, the following experiment was carried out: 50-cc. portions of fresh sea water containing 50 mgm. of $NaNO_3$ were placed in a series of large tubes and 25-gram portions of sand added. Small portions of dry algal material, using *Fucus vesiculosus* or *Ulva lactuca,* were added to some of the tubes; some cultures, with and without nitrate, were used as controls. Some of the tubes were left without further inoculation, while some were inoculated with marine mud. All the cultures were incubated at room temperature and readings taken at the end of 5 and 9 days. The water used in this experiment was taken near shore at the Woods Hole harbor, while the mud was obtained off the shore of Gay Head.

The results presented in Table VI show that algal material can be used readily as a source of energy for the reduction of nitrate by the bacteria living in the sea water and in marine mud. There were found several marked differences, however, between the different cultures, depending on the nature of the algal material and on the kind of inoculum. In the sea water medium, the organic constituents of the *Ulva* were used readily as sources of energy for the reduction of the nitrate, as shown by the rapid reduction of the nitrate to nitrite and to gaseous nitrogen within 5 days; after 9 days incubation, all the nitrate and nitrite have completely disappeared, due to their reduction to atmospheric nitrogen. When *Fucus* was added to the sea water, the reduction of the nitrate was much slower, no gas being formed and considerable nitrate and nitrite being still left after 9 days incubation. The sea water medium containing nitrate only and no additional energy source showed no trace of reduction of nitrate, either to nitrite or to gaseous nitrogen.

The cultures heavily inoculated with mud gave a totally different type of nitrate reduction. When the *Ulva* material was added to the medium, both the nitrate and nitrite disappeared in 9 days, with active gas evolution, even more rapidly than in the cultures of sea water alone. In the case of the cultures receiving the *Fucus* the reduction of the nitrate was so rapid that, within 5 days, all the nitrate and nitrite disappeared completely from the culture, accompanied by abundant gas evolution. The difference in the influence of the *Fucus* material in the cultures inoculated with water and with mud is due largely to the fact that, as suggested previously, marine mud probably contains bacteria capable of attacking the specific carbohydrates of the *Fucus,* while such bacteria are absent in the sea water itself. The mud-inoculated cultures receiving no algal material also gave active reduction of nitrate to nitrite and even to atmospheric nitrogen; in this case the small amount of available organic matter in the mud (46) was probably used as a source of energy. The traces of nitrite found in the culture which had not received any nitrate but which was inoculated with mud are possibly due to the formation of nitrite by the oxidation of the ammonia which is formed in the decomposition of the organic nitrogenous complexes in the mud.

TABLE VI

Utilization of marine algæ as sources of energy for bacteria reducing nitrate.

Tr = trace of nitrite or nitrate; 0 indicates negative test; + = positive nitrite or nitrate test; ++ and +++ = considerable nitrite or nitrate present; +++++ = maximum nitrite formation.

Source of algal material	Mud inoculum	Growth of bacteria	Gas		Nitrite		Nitrate	
			5 days	9 days	5 days	9 days	5 days	9 days
Sea water alone.......		0	0	0	0	0	0	0
Sea water and nitrate......		0	0	0	0	0	++	++
Sea water, nitrate, and Ulva.......		Turbidity in culture	+	0	+++++	0	++	tr
Sea water, nitrate, and Fucus......		Turbidity and pellicle	0	0	+++	+++++	++	+
Sea water alone	+	0	0	0	0	+	0	0
Sea water and nitrate......	+	Turbidity	+	0	++	++	++	++
Sea water, nitrate, and Ulva.......	+	Turbidity	+	+	+	0	+	0
Sea water, nitrate, and Fucus......	+	Pellicle	+	+	0	0	0	0

A study of the results of the two experiments, namely, (1) those of the cruise of the "Atlantis," which pointed to the presence in the sea of bacteria capable of reducing nitrate chiefly to nitrite, and (2) those emphasizing the importance of the specific energy source for the activities of the nitrate-reducing bacteria, will justify one in drawing certain definite conclusions. The marine algæ and members of the phytoplankton live and synthesize their cell substance in the surface layers of the water, or in the zone of maximum photosynthetic activities. As a result of these synthetic processes, the plants consume the nitrate present in the particular layers of water in the sea. The bacteria present in the water or attached to the algæ or the phytoplankton feed upon certain secretion products of these marine plants or upon the plant detritus, after the plants die. Since these algal and diatom substances are either free from nitrogen, as in the case of some of the secretion products, or contain only very limited amounts of this element, the bacteria decomposing them, unless they are nitrogen-fixing organisms, have to draw upon the available nitrogen present in the surrounding medium, namely in the sea. This results in the consumption of a certain amount of nitrate by the bacteria. As a result of these two processes, namely phytoplankton development and bacterial consumption of the algal products, the nitrate tends to disappear in the surface layers of water in the sea.

Among the bacteria attacking the algal and diatom products and residues in the surface layers of the water are present organisms which not only consume the nitrate and transform it into organic nitrogenous compounds, as constituents of the bacterial cell substance, but which also reduce the nitrate. Such reduction will take place only in the presence of an excess of nitrate, above that consumed by the algæ and diatoms and the heterotrophic marine bacteria. This reduction is carried largely to the nitrite stage, but not to atmospheric nitrogen. One would, therefore, expect that where active plant growth takes place in the sea nitrites should be present rather than nitrates. This does not indicate any loss of nitrogen from the sea but merely that at a certain level below the surface of the water, conditions are favorable to the reduction of the nitrate to nitrite. The latter can, of course, be used by the growing algæ and diatoms as a source of nitrogen just as well as the nitrate itself.

The marine bottom presents a totally different bacteriological system from that of the water itself. Not only does it harbor an extensive bacterial population capable of reducing nitrate to nitrite but it may also contain true denitrifying bacteria. However, the occurrence of such bacteria in the mud does not at all indicate that the process of complete reduction of nitrate to atmospheric nitrogen is very pronounced even at the sea bottom. Here also, certain controls prevent the rapid losses of nitrogen in a combined form which is so essential for the life of the plants in the sea. In order to reduce the nitrate (or nitrite) to atmospheric nitrogen, the denitrifying bacteria must have a source of available energy; but the organic matter of the sea bottom, or the marine humus, can be used only to a limited extent as this source of energy. This marine humus contains carbon and nitrogen in such proportions (46) that its decomposition will give rise to available nitrogen in the form of ammonia, which is used as a substrate for the bacteria producing nitrate, through the nitrite stage. The only existing possibility for denitrification to take place is in the very surface layer of the marine bottom where incompletely decomposed plant residues may offer a source of available energy for the denitrifying bacteria. It is doubtful whether even under these conditions the nitrate-consuming bacteria, decomposing these residues, are not more predominant and more active than the nitrate-destroying bacteria.

Occurrence of Nitrogen-Fixing Bacteria in the Sea

Historical

Although the ocean is usually considered to be a large reservoir of combined nitrogen, the question as to the origin of this nitrogen has frequently been raised. Some investigators believed that it is a direct result of electric discharges, while others looked to the land as the sole source of this nitrogen; however, the belief expressed by some that bacteria are responsible for the presence of combined nitrogen in the sea has been gaining ground. There is no doubt that all these three theories have some justification. The presence of a considerable amount of nitrogen in marine mud (46), especially not far from land, points to the latter as a possible source of at least some of the nitrogen present in the sea; whether this nitrogen is readily available for the nutrition of marine algæ and plankton is another question, and one which is still little understood.

The function of the nitrogen-fixing bacteria in increasing the supply of combined nitrogen in the sea is difficult to determine, the results obtained under laboratory conditions not lending themselves readily to a direct application to natural marine processes. While the importance of the bacteria concerned in the fixation of atmospheric nitrogen on land is well established, the available information about the occurrence and activities of these bacteria in the sea is still very elementary. When one considers further the fact that, of the two groups of nitrogen-fixing bacteria on land, the function of only the symbiotic organisms, namely root-nodule bacteria, is well established, while the importance of the non-symbiotic bacteria is still in dispute, and that it is the latter group which is found in the sea, one can readily understand the reason for the lack of sufficient knowledge concerning the activities of these bacteria in the sea.

Benecke and Keutner (7) were the first to demonstrate that both the ærobic *Azotobacter* and the anærobic *Clostridium pastorianum* are found in the sea, the former occurring largely on the plankton, while the latter is present chiefly in the lower layers of water and in the sea bottom. When an attempt was made to isolate these organisms, they were found to be always accompanied by other bacteria, largely two spore-forming types. Keutner (28) concluded in 1905 that nitrogen-fixing bacteria are normal inhabitants of the sea, having been demonstrated in the North Sea, in the Indian Ocean, on the African coast, and in the Malay Archipelago. The amount of nitrogen fixed per culture containing 4 grams of glucose in 200 cc. of water varied from 5.7 to 16.0 milligrams, in the presence of calcium carbonate, and from 0.9 to 9.0 milligrams in its absence.

Keding (27) found *Azotobacter* to be present in the slimy substance formed on the surface of various marine algæ; he believed that the strains of this organism found in the sea are identical to those present on land. Nathanson (37), however, could not find *Azotobacter* in the Gulf of Naples; as a result of this, he was inclined to conclude that the occurrence of this organism in the sea is merely a matter of infection with land water. Benecke (6) repeated this work and had no difficulty in establishing the common occurrence of this organism in the Gulf of Naples; the negative results obtained by Nathanson were due entirely to improper methods of study. As is usually the case, negative results are frequently no proof at all that a specific organism is absent in the sea, but may be due to a lack of a proper technique or to unfavorable conditions of culture. Typical *Azotobacter* cultures were isolated by Benecke from mud taken at a depth of 20 to 100 meters.

The fact that *Azotobacter* occurs abundantly on marine algæ led Reinke (40, 41) to suggest that one deals here with a case of symbiosis, similar to that of leguminous plants, the algæ supplying *Azotobacter* with carbohydrates as sources of energy and using the nitrogen fixed by the latter. Issatchenko (24, 25) also demonstrated the presence of typical strains of *Azotobacter* and *Clostridium* in media inoculated with some fresh *Fucus*. When the medium was free from salt no development took place, tending to indicate that one deals here with organisms specifically adapted to sea water. The presence of both *Azotobacter* and *Clostridium* was established by Bavendamm (4) in the calcareous mud of various parts of the Bahama region, near Williams Island.

These results lead one to the conclusion that nitrogen-fixing bacteria are present in the sea, especially in the bottom material and in connection with the phytoplankton and marine algæ. Since these bacteria require a source of energy for their development and since the marine humus is not available for this purpose, one would naturally expect to find them in places where energy is made available either through the growth of the chlorophyl-bearing forms of life or after their death.

Experimental

The following experiments deal largely with the methods to be used for demonstrating the presence of nitrogen-fixing bacteria in the sea. In all cases the organisms must be allowed to develop on a favorable enrichment medium, containing an available source of energy and the necessary minerals which are either lacking in sea water or are present there in limited amounts. A presumptive test for the presence of non-symbiotic nitrogen-fixing bacteria in the enriched culture is the demonstration, by means of microscopic preparations, of the characteristic cells of the ærobic *Azotobacter* or the anærobic *Clostridium pastorianum.* Confirmative tests are made by determining the increase in total nitrogen content of the medium; for full confirmation, however, the organism must be isolated in pure culture and its ability to increase the total nitrogen content of an artificial medium determined.

The first experiment was largely of a preliminary nature, namely on the influence of the energy source upon the development of nitrogen-fixing bacteria in sea water media. One hundred-cc. portions of fresh sea water containing 0.05 per cent of K_2HPO_4 were placed in a series of flasks and 1 per cent quantities of various energy sources added. Some of the flasks received 40 grams of moist sand and others did not. When glucose and mannitol were used as sources of energy, abundant gas formation and butyric acid development took place, within 4–10 days in the flasks containing sand; this pointed to the development of anærobic butyric acid bacteria. The sand-free flasks gave some growth, which was not typical, however, of nitrogen-fixing bacteria. In view of the fact that no $CaCO_3$ was added to the medium and that the reaction was changed to pH 5.4–4.4, due to acid production, the lack of *Azotobacter* development could be readily understood. When calcium acetate was used as a source of energy, active development of the anærobic *Clostridium* was accompanied by the growth of *Azotobacter;* the final reaction of this medium was pH 7.4. Sterilization of the medium followed by inoculation with fresh sea water did not influence the development of the nitrogen-fixing organisms.

In view of the importance of iron in the process of fixation of nitrogen by *Azotobacter,* an iron-ligno protein compound (49) was also added. The final basic medium adopted for these investigations consisted of:

1000 cc. sea water
0.5 gram K_2HPO_4
0.5 gram iron-ligno-proteinate
20 grams glucose, mannitol, or Ca acetate.

The purpose of the next experiment was to establish the ability of the mixed bacterial population of the sea to fix nitrogen. One hundred cubic centimeter portions of the above medium, prepared from fresh sea water, were placed in a series of 250-cc. Erlenmeyer flasks. Some of the flasks received 40-gram portions of sand, 2–3 grams of $CaCO_3$ and 2 grams of the energy source; some flasks received only $CaCO_3$ and the energy source, while some did not receive any $CaCO_3$. The cultures were incubated for 32 days and the nitrogen determinations made.

The results presented in Table VII prove that sea water contains active nitrogen-fixing organisms. The presence of sand is not necessary to bring about their optimum activities. Glucose and mannitol were found to be usually better sources of energy than Ca acetate. The addition of $CaCO_3$ is necessary, when sugars are used, to neutralize the acidity produced by the anærobic organisms; the presence and activities of these are indicated by the abundant gas evolution. The crude cultures fixed appreciable quantities of nitrogen. The fixation of nitrogen was greatest in the presence of $CaCO_3$, except in the case of those cul-

TABLE VII

Nitrogen fixation in sea water with and without sand, with different carbon sources. Two grams of energy source used per culture. Cultures were incubated 32 days.

Sand present	Energy source	$CaCO_3$ added	Gas formation	Nitrogen fixed per culture
				mg.
–	Glucose		0	11.2
–	Glucose	+	0	12.5
–	Mannitol		+	0.2
–	Mannitol	+	+	13.8
–	Ca acetate		+	0.9
–	Ca acetate	+	Trace	2.0
+	Glucose		+	3.7
+	Glucose	+	0	8.2
+	Mannitol		+	4.8
+	Mannitol	+	0	8.6
+	Ca acetate		0	3.1
+	Ca acetate	+	0	4.6

tures where lack of gas evolution pointed to the unfavorable conditions for the development of the anærobic bacteria; a good pellicle development of *Azotobacter* and accompanying forms always indicated abundant fixation of nitrogen.

In order to test the presence and distribution of nitrogen-fixing bacteria in the sea, two types of media were used, one favorable for the development of anærobic bacteria and one for the growth of ærobic organisms: 1. The medium previously described was prepared, and 35-cc. portions, together with 10 grams of dry washed sand, were placed in large test tubes and sterilized for 15 minutes at 15 lbs. pressure; this medium was particularly favorable for demonstrating the presence of the anærobic *Clostridium* in sea water and in sea bottom; the relative abundance of this organism was determined by the dilution procedure. 2. The silica-gel plate was utilized for the purpose of demonstrating the presence of ærobic nitrogen-fixing bacteria in the sea.

The plates containing the gel (47) are placed in flowing tap water for 12–16 hours, to remove excess of chloride; they are then removed from the tap water and immersed in freshly boiled and cooled sea water, for 5–10 minutes. The excess of water is drained off and carefully dried off and the plates covered with sterile tops. A nutrient solution [4] is prepared so that when 2 cc. of this solution is added to a 100-cc. portion of silica gel, using a plate 15 cm. in diameter, the final medium will contain 2 per cent of the carbon source. Distilled water is used for the preparation of the nutrient solution. The plates are then exposed in a drying oven kept at 60° C. for a few minutes, until the excess of water has been removed. A thin layer of sterile $CaCO_3$ is powdered on the surface of the medium, the plates are covered with sterile tops, and are ready for inoculation. Special care must be taken in the preparation of this medium, since an excess of salt or too thin a layer of medium will result in the rapid drying out of the gel and will prevent bacterial development.

The cruise of the " Atlantis " in August, 1932, to the Gulf of Maine and George's bank (50) presented a good opportunity for determining the presence of ærobic and anærobic nitrogen-fixing bacteria on the high seas, both in the sea water and in the mud. A series of tubes with sterile liquid medium and a series of plates with the silica-gel medium were taken aboard ship and inoculated with water and mud from the various stations, immediately after the samples were taken. It was demonstrated in a previous contribution (50) that there is a definite parallelism between the total numbers of bacteria in the water, as determined by the plate method, and the number of plankton organisms;

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[4] This solution also contained 0.05 per cent K_2HPO_4 and 0.05 per cent iron lignoprotein complex.

this was especially true of the phytoplankton. Whether the occurrence of *Azotobacter* in the sea has also a certain relation to the phytoplankton still remains to be determined. At the time of the cruise, there was comparatively little phytoplankton in the water. This may offer the explanation for the fact that not all the stations, especially the free water, gave evidence of *Azotobacter* growth. It may also be due to the fact that the silica gel medium used in this experiment was not quite favorable for the growth of the organism, as found later when the details of the method were better understood.

In the case of one station (1329), the surface layer of the marine mud gave an excellent growth of the typical *Azotobacter chroöcoccum*, using the silica gel plate. The liquid cultures showed marked differences, when water and mud were used as inocula. When 1 cc. of surface water was added to the medium, good pellicle development took place; the pellicle contained typical *Azotobacter* cells, which were also accompanied by various other bacteria. In the case of one station (1331), samples of water were taken, by means of sterile glass tubes, at the surface of the water and at depths of 30, 50, 100, and 215 meters. Only the surface and the 50 meter samples gave pellicle formation on the liquid medium; the other samples gave no signs of growth. Very few of the samples of free sea water, obtained from the different stations, brought about any gas formation or butyric acid production in the liquid medium, thus proving the absence of the anærobic *Clostridium* in the free water.

TABLE VIII

Fixation of nitrogen by crude culture of Azotobacter chroöcoccum. Two-gram energy source.

Energy source	Organism	Medium	Incubation	Nitrogen fixed per culture
			days	*mg.*
Glucose	Control	Silica plate	21	0.1
Glucose	Azotobacter	Silica plate	21	5.4
Mannitol	Azotobacter	Liquid medium	15	6.4
Glucose	Azotobacter	Liquid medium	17	4.8

The liquid media inoculated with the mud samples gave abundant gas formation, the medium itself becoming decidedly turbid. A microscopic examination of the cultures showed the presence of *Clostridium* cells, especially when glucose was used as a source of energy. This organism was always accompanied by other bacteria, usually small and large rods, some of which were spore-forming organisms.

These results suggest that anærobic nitrogen-fixing bacteria of the

Clostridium pastorianum type are universally distributed in the sea bottom, but are present only to a very limited extent in the sea water. The ærobic *Azotobacter* is also found in the sea, but much less frequently than the anærobic *Clostridium*. Whether *Azotobacter* is less abundant in the sea or whether the media used for demonstrating its presence were not always sufficiently favorable for its development remains to be determined. Although there is no doubt that *Clostridium* finds its natural medium in the sea bottom, it still remains to be established whether *Azotobacter* is normally present in the sea water, in the plankton, on the surface of algal growth, or in the sea bottom. Benecke and Keutner, Issatchenko, and others found this organism on the surface of marine algæ and suggested, therefore, its symbiotic existence with the latter; however, its presence on the bottom of the sea suggests also the possibility of its free existence, whereby the remains of algal and plankton growth are used as sources of energy.

Various other bacteria were encountered in the nitrogen-free media, either accompanying *Clostridium* and *Azotobacter*, or even without these organisms; they consisted of different cocci and small rods, occasionally thread-like forms. Whether these bacteria are able to fix nitrogen or whether they play any part in this process at all still remains to be determined.

In order to demonstrate the ability of the culture of *Az. chroöcoccum* obtained from the bottom mud of Station 1329, by the use of the glucose-silica-gel plate, to fix atmospheric nitrogen, the total nitrogen content of the gel in which this organism was growing was compared with that of a control plate. The crude culture of this organism was also inoculated into a 100-cc. portion of liquid medium containing 2 per cent of glucose or mannitol, incubated for 15–17 days and the amount of nitrogen fixed determined (Table VIII). The results obtained prove beyond any doubt the ability of the *Azotobacter* found in the sea bottom to fix considerable quantities of nitrogen in sea water media, in the presence of available sources of energy.

The *Azotobacter* was later isolated free from the accompanying bacteria, as shown in Fig. 3. However, in view of the fact that the nitrogen-fixing organisms do not live in the sea in the form of pure cultures, but are present there as mixed populations, a knowledge of their ability to fix nitrogen in such a mixture is more important than that of a pure culture.

Figures 1 to 8 show a group of preparations of bacteria isolated from the nitrogen-fixing cultures in which sea water and bottom material of the Gulf of Maine were used. The *Azotobacter* produced on the silica gel plate a typical growth of *Az. chroöcoccum*, forming at first a yellow pigment which later turned deep brown to black. The *Clostridium* produced abundant gas from sugars in liquid media. The ability of the three small bacterial forms shown in Figs. 6, 7, and 8 to fix nitrogen has not been determined. They are common types which develop on nitrogen-free media inoculated with sea water or with sea bottom material.

Although the results presented here are to be considered as preliminary in nature, they are quite sufficient to bring out the fact that nitrogen-fixing bacteria, both of the ærobic and anærobic types, are commonly found in the sea. There is no doubt that in the presence of an available source of energy and other nutrients, such as available iron and phosphate, they become active in the sea and are able to fix atmospheric nitrogen.

A study of the relation of these bacteria to the phytoplankton and plankton residues on the sea bottom, the natural sources from which they derive their energy, would necessitate a study of the occurrence of *Azotobacter* at certain stations throughout the year. This, as well as further study of the relation of the nitrogen-fixing bacteria to the available nutrients in the sea, changing conditions, season of year, depth, distance from shore, etc., are still problems awaiting further investigation. For this purpose, the silica gel plate method seems to be ideal for the determination of the occurrence of *Azotobacter,* while the liquid-sand culture in the tube is very convenient for studying the abundance of *Clostridium.*

SUMMARY

1. A preliminary study has been made of the occurrence and activities in the sea of bacteria concerned in the cycle of nitrogen in marine life.

EXPLANATION OF PLATE I

FIG. 1. Mixed culture of *Azotobacter* and accompanying bacteria. Mud of Station 1329 inoculated on silica gel plate. × 1031.

FIG. 2. Culture of *Azotobacter* on liquid media, isolated from sea water, accompanied by short rod-shaped bacteria. × 1031.

FIG. 3. Pure culture of *Azotobacter* isolated from sea water. × 1031.

FIG. 4. Culture of *Azotobacter* isolated from mud of Station 1329 on silica-gel media and cultivated on nitrogen-free glucose media, showing granulation. × 1031.

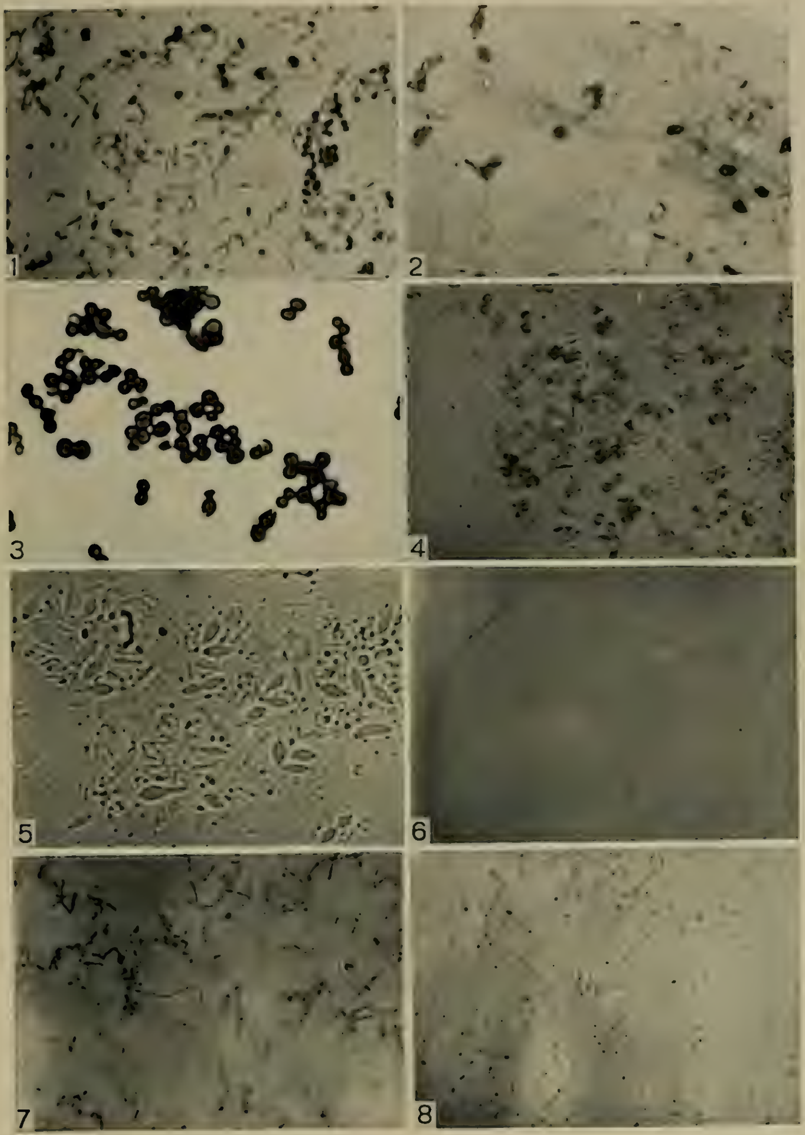
FIG. 5. Culture of *Clostridium* isolated from marine mud. Culture impure, unstained preparation. × 1031.

FIG. 6. Thread-forming bacteria growing in nitrogen-free glucose media inoculated with marine mud. × 1031.

FIG. 7. Spore-forming rod-shaped bacteria growing on nitrogen-free media. × 1031.

FIG. 8. Spore-forming rod-shaped bacteria isolated from surface water in the Gulf of Maine (Station 1331) and grown on nitrogen-free mannitol media. × 1031.

Plate I



2. Sea water, especially in the upper layers, is practically free from nitrifying bacteria or contains only very few cells. The activities of these organisms are limited almost entirely to the sea bottom.

3. In order to demonstrate the presence of these organisms even in the bottom material, it is essential to use a medium containing a layer of sand or mud, so as to make conditions favorable for their development.

4. It is comparatively easy to demonstrate the presence in the sea bottom of bacteria oxidizing ammonium salts to nitrite. It is somewhat more difficult to establish the presence of nitrate-forming bacteria; this can be done, however, if the sensitiveness of these organisms to the laboratory conditions is taken into consideration, especially nutrient concentration and period of incubation.

5. Sea water and sea bottom contain an abundance of nitrate-reducing bacteria. Most of these organisms, especially those present in the water, are able, however, to reduce the nitrate only to nitrite, but not to atmospheric nitrogen.

6. Bacteria that bring about complete reduction of the nitrate are present largely in the sea bottom. In order to reduce the nitrate, an available source of energy must be present. The marine humus cannot be used to any great extent as such a source of energy. The plankton residues are used largely by those bacteria which consume the nitrate and transform it into organic nitrogenous compounds.

7. The conclusion is reached that, even if denitrifying bacteria are present in the sea, their activities are so limited under marine conditions as to render them in most instances of little importance in limiting the nitrate supply of the sea.

8. The sea contains an abundant population of nitrogen-fixing bacteria. The ærobic *Azotobacter* is largely found in the water and in the surface layer of the marine bottom, while the anærobic *Clostridium* is present in the sea bottom.

9. In the presence of a favorable source of energy, the marine nitrogen-fixing bacteria are capable of fixing appreciable quantities of nitrogen. It still remains to be determined, however, to what extent this process actually takes place in the sea.

10. The bacteria concerned in the transformation of nitrogen can be tentatively distributed among the plankton and the sea bottom, the two major seats of bacterial activities in the sea, as follows: the nitrifying bacteria live largely on the sea bottom; of the nitrate-reducing bacteria, those forms which reduce nitrate to nitrite live both in the water and in the bottom, while the true denitrifying bacteria live largely on the bottom; of the nitrogen-fixing bacteria, the ærobic forms live both in the

water and on the bottom, while the anærobic forms live largely in the bottom material.

BIBLIOGRAPHY

1. Audoynaud, A., 1875. Recherches sur l'ammoniaque contenue dans les eaux marines et dans celles des marais salants du voisinage de Montpellier. *Compt. Rend. Acad. Sci.,* **81:** 619.
2. Baur, E., 1902. Ueber zwei denitrifizirende Bakterien aus der Ostsee. *Wiss. Meeresunters. Kiel,* N. F., **6:** 9.
3. Bavendamm, W., 1931. The Possible Rôle of Micro-organisms in the Precipitation of Calcium Carbonate in Tropical Seas. *Science,* **73:** 597; *Ber. deutsch. bot. Gesell.,* **49:** 282.
4. Bavendamm, W., 1932. Die mikrobiologische Kalkfällung in der tropischen See. *Arch. Mikrob.,* **3:** 205.
5. Beijerinck, M. W., 1890. Over lichtvoedsel en plastisch voedsel van lichtbacterien. *Meddel, K. Acad. Wetensch.,* **7:** 239.
6. Benecke, W., 1907. Über stickstoffbindende Bakterien aus dem Golf von Neapel. *Ber. deut. bot. Gesell.,* **25:** 1.
7. Benecke, W., and J. Keutner, 1903. Über stickstoffbindende Bakterien aus der Ostsee. *Ber. deutsch. bot. Gesell.,* **21:** 333.
8. Berkeley, C., 1919. A Study of the Marine Bacteria, Straits of Georgia, B. C. *Trans. Roy. Soc. Canada,* **13** (V) : 15.
9. Brandt, K., 1899. Ueber den Stoffwechsel im Meere. *Wiss. Meeresunters. Kiel,* N. F., **4:** 213; 1902, **6:** 23; 1916–1920, **18:** 185; 1919, **19:** 251.
10. Brandt, K., 1904. Über die Bedeutung der Stickstoffverbindungen für die Produktion im Meere. *Bot. Centrbl. Beih.,* **16:** 383.
11. Brandt, K., 1923–27. Stickstoffverbindungen im Meere. I. *Wiss. Meeresunters. Kiel,* **20:** 201.
12. Butkewitch, W. C., 1932. Method of Bacteriological Investigation and Certain Data on the Distribution of Bacteria in the Water and Bottom of Barents Sea. *Trans. Russian Oceanogr. Inst.,* **2:** No. 2.
13. Drew, G. H., 1914. On the Precipitation of Calcium Carbonate in the Sea by Marine Bacteria and on the Action of Denitrifying Bacteria in Tropical and Temperate Seas. *Papers from Tortugas Laboratory, Carnegie Inst. Washington,* **5:** 7; 1913, *Jour. Mar. Biol. Ass.,* **9:** 479.
14. Feitel, R., 1903. Beiträge zur Kenntnis denitrifizirender Meeresbakterien. *Wiss. Meeresunters. Kiel,* **7:** 89.
15. Fischer, B., 1894. Die Bakterien des Meeres nach den Untersuchungen der Plankton-expedition unter gleichzeitiger Berücksichtigung einiger älterer und neuerer Untersuchungen. *Centrbl. Bakt.,* **15:** 657; 1894, Ergebnisse der Plankton-expedition der Humbolt stiftung., **4:** m. g. Verlag. von Lipsius Tischer. Kiel, Leipzig.
16. Gebbing, J., 1910. Über den Gehalt des Meeres an Stickstoffnährsalzen. *Intern. Rev. Ges. Hydrob.,* **3:** 50.
17. Gräf, Dr., 1909. Forshungsreise S.M.S. Planet 1906–1907, **4:** Biologie.
18. Gran, H. H., 1901. Studien über Meeresbakterien. I. Reduction von Nitraten und Nitriten. *Bergens Mus. Aarb.,* **10:** 1.
19. Gran, H. H., 1903. Bacteria of the Ocean and their Nutrition. *Naturen. Bergen,* **27:** 33.
20. Gran, H. H., 1912. Pelagic Plant Life. In Murray and Hijort's "The Depths of the Ocean." London, pp. 307–386.
21. Harvey, H. W., 1925. Oxidation in Sea Water. *Jour. Mar. Biol. Ass.,* **13:** 953.

22. Harvey, H. W., 1926. Nitrate in the Sea. *Jour. Mar. Biol. Ass.,* **14**: 71.

23. Harvey, H. W., 1928. Biological Chemistry and Physics of Sea Water. Cambridge Univ. Press.

24. Issatchenko, B. L., 1908. Zur Frage von der Nitrifikation in den Meeren. *Centrbl. Bakt.,* II, **21**: 430.

25. Issatchenko, B. L., 1914. Recherches sur les microbes de l'ocean Glacial Arctique. Petrograd.

26. Issatchenko, B., 1926. Sur la nitrification dans les mers. *Compt. Rend. Acad. Sci. Paris,* **182**: 185.

27. Keding, M., 1906. Weitere Untersuchungen über stickstoffbindende Bakterien. *Wiss. Meeresunters. Kiel, N. F.,* **9**: 273.

28. Keutner, J., 1905. Ueber das Vorkommen und die Verbreitung stickstoffbindender Bakterien im Meere. *Wiss. Meeresunters Kiel, N. F.,* **8**: 27.

29. Kühl, H., 1908. Beitrag zur Kenntnis des Denitrifikationsprozesses. *Centrbl. Bakt.,* II, **20**: 258.

30. Liebert, E., 1915. Über mikrobiologische Nitrit- und Nitratebildung im Meere. *Rapp. Verhandl. Rijksinst. Visschereijonderzoek.* I (3).

31. Lipman, C. B., 1922. Does Nitrification Occur in Sea Water? *Science,* **56**: 501.

32. Lipman, C. B., 1924. A Critical and Experimental Study of Drew's Bacterial Hypothesis on $CaCO_3$ Precipitation in the Sea. *Carnegie Inst. Washington, Dept. Marine Biol.,* **19**: 179.

33. Lipman, C. B., 1926. The Concentration of Sea Water as Affecting its Bacterial Population. *Jour. Bact.,* **12**: 311.

34. Lipman, C. B., 1929. Further Studies on Marine Bacteria with Special Reference to the Drew Hypothesis on $CaCO_3$ Precipitation in the Sea. *Carnegie Inst. Washington,* **26**: 231.

35. Lloyd, B., 1931. A Marine Denitrifying Organism. *Jour. Bact.,* **21**: 89.

36. Natterer, K., 1892. *Monatschr.,* **13**: 873–896, 897–915; 1893, **14**: 624–673; 1894, **15**: 530–595; 1895, **16**: 405–581; 1899, **20**: 1–263; 1900, **21**: 1037–1060.

37. Nathanson, A., 1906. Ueber die Bedeutung vertikaler Wasserbewegungen für die Produktion des Planktons im Meere. *Abh. Matt. Phys. Kl. Kgl. Sächs. Ges. Wiss.,* **29**: 335.

38. Parlandt, D. A., 1911. On Several Denitrifying Bacteria from the Baltic Sea. *Bull. Jard. Bot. St. Petersburg.*

39. Rao, G. G., and N. R. Dhar, 1931. Photosensitized Oxidation of Ammonia and Ammonium Salts and the Problem of Nitrification in Soils. *Soil Sci.,* **31**: 379.

40. Reinke, J., 1903. Die zur Ernährung der Meeres-Organismen disponiblen Quellen an Stickstoff. *Ber. deutsch. botan. Gesell.,* **21**: 371.

41. Reinke, J., 1903. Symbiose von Volvox und Azotobacter. *Ber. deut. bot. Gesell.,* **21**: 481.

42. Russell, H. L., 1892. Bacterial Investigation of the Sea and its Floor. *Bot. Gaz.,* **17**: 312.

43. Schloesing, A., 1875. Sur l'ammoniaque de l'atmosphère. *Compt. Rend. Acad. Sci.,* **80**: 175.

44. Thomsen, P., 1907. Über das Vorkommen von Nitrobakterien im Meere. *Ber. deut. bot. Gesell.,* **25**: 16–22; 1910, *Wiss. Meeresunters. Kiel, N. F.,* **11**: 1–27.

45. Vernon, H. M., 1898. The Relations between Marine Animal and Vegetable Life. *Mitt. Zool. Sta. Neapel.,* **13**: 341.

46. Waksman, S. A., 1933. On the Distribution of Organic Matter in the Sea Bottom and the Chemical Nature and Origin of Marine Humus. *Soil Sci.,* in press.

47. Waksman, S. A., and C. L. Carey, 1926. The Use of the Silica Gel Plate for Demonstrating the Occurrence and Abundance of Cellulose-decomposing Bacteria. *Jour. Bact.,* **12**: 87.
48. Waksman, S. A., C. L. Carey, and H. W. Reuszer, 1933. Marine Bacteria and their Rôle in the Cycle of Life in the Sea. I. Decomposition of marine plant and animal residues by bacteria. *Biol. Bull.,* **65**: 57.
49. Waksman, S. A., and K. R. N. Iyer, 1932. Contribution to our Knowledge of the Chemical Nature and Origin of Humus. I. *Soil Sci.,* **34**: 43.
50. Waksman, S. A., H. W. Reuszer, C. L. Carey, M. Hotchkiss, and C. R. Renn, 1933. Bacteriological Investigations of Sea Water and Marine Bottoms in the Gulf of Maine *Biol. Bull.,* **64**: 183.
51. ZoBell, C. E., 1933. Photochemical Nitrification in Sea Water. *Science,* **77**: 27.

LIGHT INTENSITY AND THE EXTENT OF ACTIVITY OF LOCOMOTOR MUSCLES AS OPPOSED TO CILIA

JOHN H. WELSH

Zoölogical Laboratory, Harvard University, and the Woods Hole Oceanographic Institution[1]

I

The effect of light on animal behavior has attracted the attention of biologists for many years. One phase of the general problem, namely the photokinetic effect of light, has been studied to a less extent than orientation and the factors determining the phototropic condition of organisms and many questions regarding this phase of photic behavior remain to be answered. The first and most important of these is whether or not intensity of illumination does actually influence the speed at which an animal moves toward or away from a source of illumination. Some of the evidence has already been reviewed by the author (Welsh, 1932*a*). In the same paper and in additional work it has been shown that in a light-sensitive organism, which moves by means of muscles, the extent of muscular activity bears a very definite relationship to intensity of illumination. This is not in agreement with work of Mast (1923), and Dolley (1917), but does agree with the results of most investigators and seems reasonable in the light of what we know regarding the response of the receptor-muscle system to light. It is not as evident that light intensity has a similar effect on organisms which move by means of cilia or flagellæ. Observations on such forms indicate that there is no effect of luminous intensity on speed of progression. Directional light, however, does influence orientation in these animals and it is difficult to determine whether slight changes in rate are due to deviations from a straight path or to changes in amplitude and frequency of ciliary movement. It seemed desirable to search for forms which move by means of cilia and which, after primary orientation, proceed in a straight line. A study of such forms should indicate whether or not their speed of progression is a function of the intensity of illumination.

In the course of some work at the Woods Hole Oceanographic Institution there was an opportunity for such an investigation and a survey was made of the effect of light intensity on the activity of a number of marine invertebrates. The results indicate that the extent of ciliary activity is not a function of the amount of light energy reaching the organism as is true of the activity of locomotor muscles.

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[1] Contribution No. 21 from the Woods Hole Oceanographic Institution.

II

Among the animals used in the investigation were two species of small marine turbellarians, one of which was identified as *Plagiostomum* sp.; the other has not yet been identified. These were obtained largely from *Ulva* where they were found moving over the surface of the alga. They both orient by means of muscles but after primary orientation cilia alone seem to be concerned in locomotion. One important difference made these animals particularly valuable for purposes of comparison. *Plagiostomum* tends to be free-swimming and the path through which it moves is not straight, while the unidentified form always moved in contact with the substrate and its path was a nearly perfect straight line. *Columbella lunata,* a small gastropod, also moves by means of cilia. Orientation again involves muscles but they appear to play no part in forward locomotion. *Arenicola* larvæ were also used but the spiralling of this form made it difficult to distinguish between orientation effects and any change in extent of ciliary activity.

Among the animals observed which move by means of appendages operated by muscles were larvæ of *Pinnotheres maculatus, Hippa talpoida,* and stomatopod larvæ, probably of *Squilla empusa.* The copepod *Centropages typicus* and small specimens of *Limulus polyphemus* were also used. All of these forms, at the age used, were positive to light with the exception of *Limulus* which was negative.

The experiments were carried on under carefully controlled conditions. The animals were placed individually in a glass trough, 30 cm. in length, with the bottom marked in centimeters. This trough was partially submerged in a water bath maintained at 21.3° C. Light reached the animals through windows in opposite sides of the tank. The light sources were two ribbon filament lamps arranged to give an even illumination of nearly parallel rays in the mid-region of the trough, the illumination from one lamp being 1500 foot candles at this point and from the other 7.5 foot candles. These intensities were selected after numerous tests on different species had shown that the intensity of 1500 foot candles was higher than that necessary to elicit a maximum speed, while 7.5 foot candles fell within the range of intensities to which these same organisms showed a change in speed of progression (*e.g. Pinnotheres* larvæ, Welsh, 1932*a*; *Unionicola,* Welsh, 1932*b*).

Both lamps were operated from a three-way switch so that one was turned off as the other was turned on.

The animal under observation was adapted to the temperature of the sea water in which it was placed and then timed while traversing the middle 10 cm. of the trough. Ten trials were made for a given animal at each of the two light intensities, and the times were averaged. The results are shown in the following section.

III

For an analysis of the findings the animals may be divided into three groups according to their methods of locomotion and the extent to which orientation may affect the apparent velocity of forward progression. We may place in the first group those animals which move by means of locomotor appendages operated by muscles; in the second group animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light; and in the third group animals moving by means of cilia and progressing in a straight line in directional light.

TABLE I

The effect of light intensity on the speed of forward progression of animals moving by means of locomotor appendages operated by muscles.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Pinnotheres larva	6.6	±0.13	14.1	±0.47
Hippa larva	4.5	±0.06	11.1	±0.16
Squilla larva	4.9	±0.45	11.3	±0.66
Limulus	5.4	±0.05	7.4	±0.25
Centropages	4.4	±0.22	8.5	±0.77

Table I gives the average times, with their probable errors, for the first group. The results indicate that light intensity greatly influences the speed, as the times for travelling 10 cm. at the lower intensity are over twice as great as those at the higher intensity in all instances but one. This indicates that the frequency and amplitude of movement of the locomotor appendages must increase with an increase in intensity, as is true for *Unionicola* (Welsh, 1932*b*). This may be accounted for by assuming a more rapid decomposition of photo-sensitive material in the photoreceptor as shown by Hecht (1920), followed by a greater frequency of discharge over sensory tracts from the eye (Hartline and Graham, 1932), and finally more muscle fibers of the locomotor appendages being stimulated by discharge of motor nerves. Throughout this system thresholds of receptors, conductors, and effectors may be involved as well as frequency of discharge of the nerves, and it is readily apparent that the amount of light energy reaching the organism might well be expressed by the degree of activity of locomotor structures, and hence the speed at which the animal travels.

Table II gives the times for traversing 10 cm. for three different animals belonging to a species of *Plagiostomum,* and for a larva of the lug-worm *Arenicola.* In both forms cilia are involved in forward locomotion but there are considerable deviations from a straight path even in progression toward a light of high intensity. The times for travelling 10 cm. at the lower intensity are greater than those for 1500 foot candles, but in the case of *Plagiostomum* the difference is not large and there is no agreement between the average times and their probable errors as is shown in Table I. The probable error in each instance is less for the lower intensity than for the higher. It seems in the case of *Plagiostomum* that the difference in times at the two intensities might be accounted for by the deviations from a straight path which are greater at the lower of the two intensities. These deviations involve muscular bending of the body which helps in no apparent way with forward progression.

TABLE II

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Plagiostomum (1)	52.6	±2.83	61.8	±2.75
Plagiostomum (2)	33.9	±0.84	37.7	±0.65
Plagiostomum (3)	35.8	±1.80	42.6	±1.03
Arenicola larva	78.5	±1.02	123.5	± 5.6

In the case of the *Arenicola* larva the difference in times at the two intensities is proportionally greater than that for *Plagiostomum* but the deviations from a straight path were more pronounced. At the lower intensity the degree of spiralling increased and also an up and down movement was more noticeable.

Now if we examine the results from another turbellarian and from *Columbella lunata* (Table III), it is seen that intensity of illumination has no effect on the speed of progression. In these forms, as stated previously, muscles play an important part in orientation and the angle of turning bears a definite relationship to the luminous intensity. After orientation, however, the movement toward the light was a smooth, gliding one, and the deviations from a straight path were negligible. While there was considerable variation in the speed of different animals of the same species, the speed of any particular specimen was quite uniform, and this was true irrespective of light intensity.

These results would indicate that the beat of cilia is not affected by light intensity as is the frequency and amplitude of movement of locomotor muscles. These two types of effectors are quite different in their behavior to stimuli other than light, and there is no known mechanism whereby the amount of light energy reaching the photoreceptors might be realized by the cilia except in an indirect way.

TABLE III

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and progressing in a straight line in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Turbellarian sp. ? (1)	49.5	±0.57	50.1	±0.50
Turbellarian sp. ? (2)	89.1	±2.63	89.6	±2.48
Turbellarian sp. ? (3)	37.9	±0.28	37.4	±0.44
Columbella	80.2	±2.15	81.2	±2.42

IV

The results obtained on animals progressing by means of cilia are in agreement with those of Holmes (1903), and Mast and Gover (1922) obtained on flagellated forms, but they indicate that the laws governing these forms cannot be applied to forms such as the arthropods which move by means of appendages operated by muscles.

In the case of planaria there is an apparent effect of light on speed of progression (Parker and Burnett, 1900; Pearl, 1903; Walter, 1907), but in these animals, according to Stringer (1917), locomotion is essentially a muscular act in which cilia play no necessary part. Evidently only in the smaller turbellarians do cilia function as important locomotor structures. This is probably also true of the gastropods.

The observations of Umeda (1927), Wharton (1931), and Lucas (1933), as reported by Lucas, concerning the effect of light on ciliated epithelium, are of interest in this consideration although they are somewhat contradictory. Lucas (1933) found that in ciliated epithelium from the frog, quiescent cilia were sometimes made active by illumination. He reports in the same paper that Umeda obtained similar results from illumination with light of long wave lengths. Wharton (1931), however, in studying cilia of the tracheae, found that light had an apparent inhibitory effect on the beat of the cilia. As the present writer is not familiar with the experimental technique used by these investigators it is not possible to comment on the results. It is difficult to conceive of light, dissociated entirely from heat, having a direct effect on the activity of cilia of ciliated epithelium although such an effect may be possible. This problem would seem to be worthy of further investigation.

It seems safe to conclude from the evidence at hand that the activity of locomotor muscles of certain animals, and hence the speed of progression of these forms, is a definite function of the intensity of illumination over a given range, while the activity of cilia, and speed of animals moving by means of cilia bears no such relation to light intensity.

SUMMARY

The results obtained from a study of the effect of intensity of illumination on the speed of progression of a number of marine invertebrates indicate that the activity of locomotor appendages, operated by muscles, is a function of the luminous intensity and that the animals move faster in light of high intensity than in light of low intensity. This, however, is not true of animals moving by means of cilia, for such animals, provided they move in a straight line in directional light, show no change in speed and hence no change in ciliary beat as the light intensity is varied.

LITERATURE CITED

Dolley, Jr., W. L., 1917. The Rate of Locomotion in Vanessa antiopa in Intermittent Light and in Continuous Light of Different Illuminations, and its Bearing on the "Continuous Action Theory" of Orientation. *Jour. Exper. Zoöl.,* 23: 507.

Hartline, H. K., and C. H. Graham, 1932. Nerve Impulses from Single Receptors in the Eye. *Jour. Cell. and Comp. Physiol.,* 1: 277.

Hecht, S., 1920. The Photochemical Nature of the Photosensory Process. *Jour. Gen. Physiol.,* 2: 229.

Holmes, S. J., 1903. Phototaxis in Volvox. *Biol. Bull.,* 4: 319.

LUCAS, A. M., 1933. Comparison of Ciliary Activity under in vitro and in vivo Conditions. *Proc. Soc. Exper. Biol. and Med.,* **30**: 501.

MAST, S. O., 1923. Photic Orientation in Insects with Special Reference to the Drone-fly, Eristalis tenax, and the Robber-fly, Erax rufibarbis. *Jour. Exper. Zoöl.,* **38**: 109.

MAST, S. O., AND M. GOVER, 1922. Relation between Intensity of Light and Rate of Locomotion in Phacus pleuronectes and Euglena gracilis and its Bearing on Orientation. *Biol. Bull.,* **43**: 203.

PARKER, G. H., AND F. L. BURNETT, 1900. The Reactions of Planarians, with and without Eyes, to Light. *Am. Jour. Physiol.,* **4**: 373.

PEARL, R., 1903. The Movements and Reactions of Fresh-water Planarians: a Study in Animal Behaviour. *Quart. Jour. Micr. Sci.,* N. S., **46**: 509.

STRINGER, C. E., 1917. The Means of Locomotion in Planarians. *Proc. Nat. Acad. Sci.,* **3**: 691.

UMEDA, T., 1927. *Acta Dermat.,* **10**: 603. (Cited by Lucas, 1933.)

WALTER, H. E., 1907. The Reactions of Planarians to Light. *Jour. Exper. Zoöl.,* **5**: 35.

WELSH, J. H., 1932*a*. Temperature and Light as Factors Influencing the Rate of Swimming of Larvæ of the Mussel Crab, Pinnotheres maculatus Say. *Biol. Bull.,* **63**: 310.

WELSH, J. H., 1932*b*. Photokinesis and Tonic Effect of Light in Unionicola. *Jour. Gen. Physiol.,* **16**: 349.

WHARTON, D. R. A., 1931. The Effects of Certain Toxic Substances on the Ciliated Epithelium of the Guinea Pig. *Am. Jour. Hyg.,* **14**: 109. (Cited by Lucas, 1933.)

CONCHOPHTHIRIUS CARYOCLADA SP. NOV. (PROTOZOA, CILIATA)

GEORGE W. KIDDER

Department of Biology, College of the City of New York

During the month of August, 1932, the writer noticed a large holotrichous ciliate in the mantle cavity of the edible clam, *Siliqua patula,* taken from the sandy beach at Seaside, Oregon. After studying its general morphology it was decided that it was a hitherto undescribed species of the genus *Conchophthirius.* Because of its characteristically branched macronucleus it will be referred to as *Conchophthirius caryoclada.*

The methods used in this study are, in general, those described in an earlier work on this genus (Kidder, 1933*a*). Observations were made on both living and stained material.

The clams were found to be only lightly infected and while from the majority two or more ciliates were obtained the material was limited. No cases of binary fission or conjugation were found.

A study of the general morphology of *C. caryoclada* shows it to be nearly oval as viewed from the dorsal or ventral side. The size ranges from 140 μ to 250 μ in length and from 90 μ to 160 μ in width. In lateral view it is extremely thin and leaf-like, the dorso-ventral axis not exceeding one-eighth that of the lateral axis. The body is covered with fine cilia originating in basal bodies arranged in closely set rows. The cilia of the anterior region are slightly more numerous and are shorter than those of the posterior region. The ciliary rows follow the contour of the body, being nearly parallel on the ventral surface. On the dorsal surface the rows of cilia converge at the cytostome somewhat in the manner of a whorl (Fig. 1).

The cytostome is small and situated in the posterior fourth of the body on the dorsal side, about midway between the lateral edges. The two edges of the cytostome are supplied with longer cilia that beat into the opening (Fig. 1). These cilia are separate and do not form a membrane. There is no deep peristomal groove opening into the cytostome as is found in *C. mytili* (Kidder, 1933*a*), but the right end of the cytostome is pointed and continues into a narrow furrow (Fig. 1, *per. f.*). This furrow proceeds to the right lateral edge in an anterior direction, spirals around the edge to the ventral side and appears to be continuous with the thin anterior field (Fig. 2, *ant. f.*) The anterior field ends on the ventral surface near the left lateral edge not far from the anterior end. This corresponds in position to the anterior fiber of *C. mytili* (Kidder, 1933*a*) and to the anterior field of *Ancistruma* (Kidder, 1933*b*) as the ciliary rows originate along this line.

The body is covered with a rather thick, clear pellicle that is semi-rigid. No body contraction was observed although the ciliate will flex somewhat in its creeping movements. The clear ectoplasm contains many rodlike bodies which appear to be trichocysts (Fig. 1, *trich.*). However, I have never been able to cause them to explode.

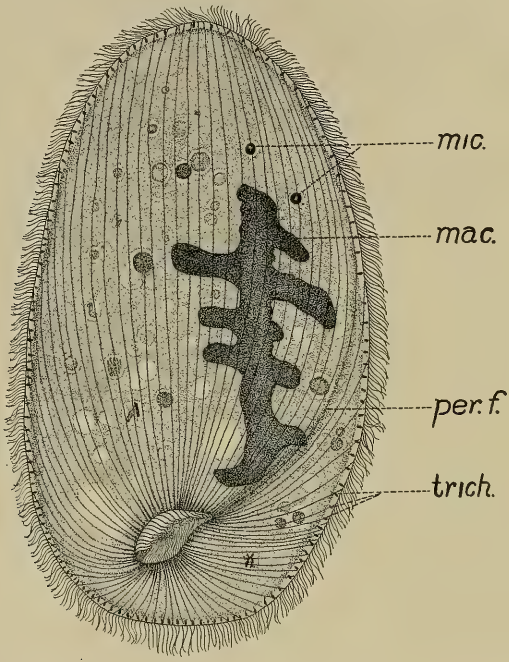


FIG. 1. *Conchophthirius caryoclada.* Dorsal view. Bouin fixation; Heidenhain's hæmatoxylin stain. × 400. Abbreviations: *mac.,* branched macronucleus; *mic.,* micronuclei; *per. f.,* peristomal furrow; *trich.,* trichocysts.

The endoplasm is finely granular and clear. Numerous food vacuoles appear in the posterior and middle region. These vacuoles are filled, for the most part, with algae taken from the water of the mantle cavity of the host. I found no host cells in these vacuoles and therefore I am inclined to regard the ciliate as a harmless commensal and not as a parasite.

Many clear vacuoles appear in the posterior region. Whether or not these are contractile vacuoles cannot be stated at this time as no contraction was observed.

The macronucleus is located in about the mid-region of the body and slightly toward the right side (Fig. 1, *mac.*). It is very branched, the branches extending laterally from the central stem. The pattern of branching varies greatly in different individuals. The macronucleus is single, all of the branches being enclosed in a continuous membrane. The chromatin is densely granular and evenly distributed.

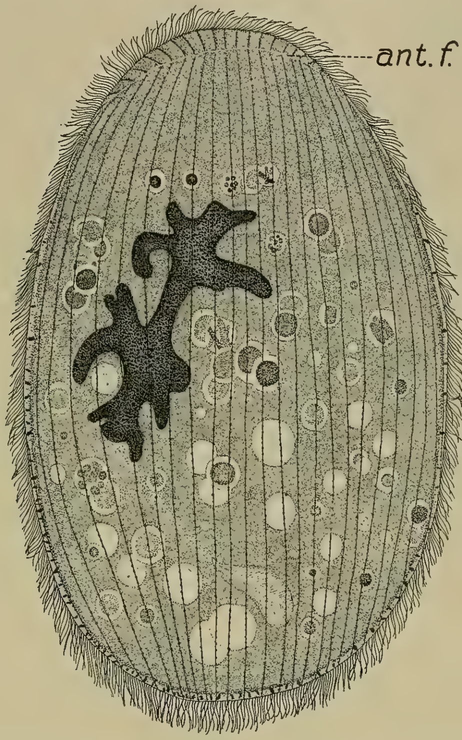


Fig. 2. *Conchophthirius caryoclada.* Ventral view. The thin anterior field, *ant. f.*, is seen at the anterior end of the body. The peristomal furrow and the cytostome are visible through the body. The chromatin of the macronucleus appears to be coarsely granular. Bouin fixation; Delafield's hæmatoxylin stain. × 400.

One or two micronuclei (usually two) are found in the anterior endoplasm near the macronucleus. They appear as dense spheres of chromatin surrounded by a clear area (Fig. 1, *mic.*). In a few forms no micronuclei could be found but this was probably due to the obscuring effect of the profusion of food inclusions.

The dorso-ventral flattening seen in the case of all members of the genus *Conchophthirius* is carried to the extreme in *C. caryoclada.* Indeed, so flat is this ciliate that its adhesive powers make it exceedingly difficult to pick up from the bottom of the glass container. The migration of the mouth has progressed much farther than in *C. mytili* so that it has come to lie on the posterior dorsal surface. This position appears to be exceedingly advantageous, as very little if any food material could be taken into a ventrally located mouth, so closely is that surface applied to the substrate.

Conchophthirius caryoclada is unique among the members of the genus in possessing a ramifying macronucleus. It is similar to those of *Rhizocaryum concavum* (Caullery and Mesnil, 1907) and *Mrazekiella nucleoramiformis* (Kijenskij, 1925). These ciliates are astomatous endoparasites. I know of no other stomatous holotrich in which the macronucleus shows this form of branching.

LITERATURE CITED

Caullery, M., and F. Mesnil, 1907. Sur l'appareil nucléaire d'un Infusoire (*Rhizocaryum concavum* n. g., n. sp.), parasite de certaines Polydores (*P. caeca* et *P. flava*). *C. R. Ass. franc. Avanc. Sc. Reims.,* **1**: 250.

Kidder, George W., 1933*a*. Studies on Conchophthirius mytili De Morgan. I. Morphology and Division. *Arch. f. Protist.,* **79**: 1.

Kidder, George W., 1933*b*. On the Genus Ancistruma Strand (Ancistrum Maupas). I. The structure and division of A. mytili Quenn. and A. isseli Kahl. *Biol. Bull.,* **64**: 1.

Kijenskij, G., 1925. Nálevníci zaživaci roury některých Oligochaetů pražskehó okolí (Morphologie, Nepohlavní množeni. Systematika). *Včstnic České Akad. Sbornik Zool.,* **75**: 1.

EFFECTS OF TEMPERATURE ON PRODUCTION OF MOSAICS IN HABROBRACON

RAYMOND J. GREB

University of Pittsburgh

It has been shown that male mosaics of *Habrobracon juglandis* (Ashmead) arise as a result of egg binuclearity, two oötids with different genes taking part in development (Whiting, 1924). Gynandromorphs also arise in a similar way except that one of the oötids is fertilized (Whiting and Stancati, 1931).

Ordinarily, mosaics occur infrequently in *Habrobracon*. While making certain linkage tests, the author found that crosses involving shot vein, sv (wings), stock females, yielded an unusually large number of mosaic males. Subsequently, this stock was used in an experiment showing that mosaicism could be increased by X-radiation (Greb, 1933).

Previous work on mosaics of other Hymenoptera has been mainly descriptive. G. A. Rösch (1926 and 1928) reported temperature experiments with the honeybee and suggested that eggs exposed to low temperatures produce an increased number of gynandromorphs. Bee breeders in general seem to agree that a lowering of the temperature in a hive will result in the appearance of a greater number of individuals mosaic for sex.

The present work was undertaken to determine whether different temperatures might not have an effect on the ratio of mosaic production in *Habrobracon,* and if so, what temperature would produce the greatest number of these individuals.

Shot vein stock was derived by mutation from Minnesota yellow, My (base of antennæ), stock during the summer of 1930 and has been kept as a pure line since that time. Since this stock gave a higher ratio of mosaics than is usually obtained, it was utilized in these experiments. Shot vein females were mated to ivory, o^1 (eyes), stumpy, st (legs), males. If, in an F_1 female, a binucleate egg occurred in which the two oötids carried different genes affecting eyes, wings, or legs, individuals from such eggs could be recognized. About half of the F_1 females from each fraternity were treated experimentally; the remainder were kept as controls. The latter were always reared at the usual breeding temperature, 30° C.

The method of procedure for the temperature experiments may be briefly summarized as follows:

Experiment A

The experimental F_1 females were subjected to a temperature of 5 to 10° C. in an electrical refrigerator for one hour. After treatment they were kept at room temperature for about an hour to allow them to recover. They were then given caterpillars and kept at 30° C. until time of transfer—four days. At this time the females were transferred into new vials, cooled as before, given time to recover and then set with caterpillars for another four days. This procedure was continued for four consecutive treatments. Offspring were reared at 30° C. Results are given in Table I.

Table I

Effects of Various Temperatures on Ratio of Mosaics

Experiment		♀♀ Set	Progeny						
			♀♀	♀͡♂	Males				
					Reg-ular	♂͡♂	Per cent ♂͡♂	Differences	Mu-ta-tions
A	Treated 5°–10° C....	78	895	2	1676	8	.4755±.1683	.0888±.2289	0
	Controls 30° C....	67	981	2	1543	6	.3885±.1583		1
B	Treated −14°–10° C.	147	1892	5	2526	9	.3563±.0799	.0336±.1106	0
	Controls 30° C.....	115	1779	4	2499	8	.3227±.0765		0
C	Treated 18°–20° C...	192	691	0	1762	0	.0000±.0000	.2675±.0831	0
	Controls 30° C...	118	1167	2	2242	6	.2675±.1090		1
D	Treated 35°–37° C...	107	1171	5	3761	38	1.0137±.1101	.7356±.1236	1
	Controls 30° C...	95	1562	3	3989	11	.2757±.0560		2
E.	Treated 35°–37° C...	144	0	0	4900	37	.7636	——	0

The symbol ♂͡♂ has been suggested to designate male mosaicism and the symbol ♀͡♀ to designate female mosaicism; ♀͡♂ = gynandromorph.

Experiment B

The treatment in Experiment B was the same as above except that the test females were subjected to — 14 to — 10° C., likewise in an electric refrigerator. This was the extreme low temperature obtainable in the ice cube chamber. The exposure was continued for one hour at times of first two treatments and for thirty minutes at last two. It had been found that a third one-hour exposure to this low temperature was too severe, killing many of the preliminary test females. Results are shown in Table I.

Experiment C

Experimental F_1 females were given caterpillars and placed in an incubator in which a temperature of 18 to 20° C. was maintained. This temperature was kept constant by placing an incubator with an electric bulb and an automatic thermostat in an electric refrigerator. Transfers were made only about every twelve days since all the activities were slowed down by the low temperature and the young larvæ did not appear until about the twelfth day. At this time the females were transferred to new vials with fresh caterpillars and returned to the low temperature incubator. The developing larvæ were reared at the usual breeding temperature (30° C.). It was possible to make, on the average, only three transfers before the females died. Consequently, more females had to be kept at this temperature in order to obtain good numbers. Results are recorded in Table I.

Experiment D

A high temperature experiment was made with conditions similar to the last experiment except that the test females were kept in an incubator in which a temperature of 35 to 37° C. was maintained. Every fourth day the females were transferred to new vials with fresh caterpillars and returned to the high temperature incubator, while the vials of larvæ were set at 30° C. Results are summarized in Table I.

DISCUSSION

Mosaicism

Data presented in Table I (Experiments *A* and *B*) show that short time treatments with very low temperatures do not appreciably change the rate of production of male mosaics or of gynandromorphs in *Habrobracon.* In case treatments were effective, we would expect both types of mosaics to vary in the same direction, since they come from binucleate eggs.

G. A. Rösch (1926 and 1928) reports results of short time cold treatments on newly laid eggs of the honeybee. By this means he obtained a number of gynandromorphs. However, he was not primarily interested in ratios, and consequently he gives no data regarding controls. Numbers of gynandromorphs vary irregularly with duration and intensity of treatment, making it impossible to derive any definite conclusions regarding effects of temperature.

Habrobracon females kept always at a low temperature of 18 to 20° C. produced no male mosaics or gynandromorphs. It was thought that constant cold might slow down maturation movement within the egg in such a way that development would more frequently start from a binucleate egg. However, this certainly did not occur. There was no evidence of development from binucleate eggs, while sister females kept at 30° C. produced usual percentages of mosaic males and gynandromorphs.

A higher temperature (35 to 37° C.) than standard, approaching the upper survival limit for *Habrobracon,* did increase the rate of production of binucleate eggs to nearly four times that found among control males (see Table I, Experiment *D,* for ratios and differences). Calculations also show that the percentage of gynandromorphs among individuals from fertilized eggs is greatest at this temperature, the ratio being significantly different from the same ratio in the control group (percentage of gynandromorphs among individuals from fertilized eggs: treated, .4269 ± .1428; controls, .1914 ± .0745; difference, .3355 ± .1218). Not a slowing down but a speeding up of maturation processes is required to obtain a large number of binucleate eggs in *Habrobracon.*

Recently another experiment was run at the same high temperature (Experiment *E,* Table I). No normal temperature control was run since the experiment was not planned to deal with ratios. However, results are consistent in showing that high temperature increases the number of individuals of binucleate egg origin. The ratio of mosaics is lower than that in Experiment *D,* probably because a new factor, honey body color, had been introduced into the ivory stumpy males.

Anna R. Whiting and Carey H. Bostian (1931) obtained a ratio of one mosaic among 1457 males (total count 6 among 8741) from diheterozygous mothers. In the experiments discussed here ratios of mosaics vary from approximately one in 260 males (Experiment *A*) to one in 370 males (Experiment *C*) in the control groups, which may be taken to indicate the range to be expected under normal conditions from the particular cross used. Temperature treatment broadens this range. Constant cold reduces the number of mosaics to zero while high temperature causes one out of about every 100 eggs to develop into a mosaic.

Percentage of gynandromorphs, as is expected, varies in the same direction as percentage of male mosaics. Among the controls, this range is one gynandromorph in 585 (Experiment *C*) fertilized eggs to one in 492 (Experiment *A*). Females exposed to constant cold produced no gynandromorphs among 691 offspring from fertilized eggs while those exposed to high temperature produced about one gynandromorph in every 235 fertilized eggs.

A comparison of the above ratios shows that there are always recorded proportionally fewer gynandromorphs than mosaic males. Both come from binucleate eggs but criteria for distinguishing the two are different. Since sex differences involve most regions of the body—antennæ, ocelli, wing size, abdominal sclerites and genitalia, as well as general body pigmentation, a gynandromorph would be more easily recognized than a male mosaic for some trait affecting a limited region such as eyes or wings. Since polyspermy occurs in eggs of Hymenoptera, fertilization of both nuclei of a binucleate egg would presumably lead to the production of a female rather than a gynandromorph.

Goldschmidt (1931), in his work on *Bombyx mori* L., obtained a large number of individuals (mosaic males, mosaic females, and gynandromorphs) which must have come from binucleate eggs in which both nuclei had been fertilized. Sex determination is different in *Bombyx* than in *Habrobracon* and both nuclei must be fertilized in order that an individual can be developed.

An effort has been made to obtain mosaic females in *Habrobracon.* Females heterozygous for shot vein, ivory, honey, and stumpy were mated to shot vein, ivory, honey, and stumpy males. It was hoped that the two oötids of a binucleate egg, differing by at least one factor, would be fertilized by recessive sperm and that a female mosaic would appear. The 69 females set produced: 692 females, 2 gynandromorphs, 880 males, and 2 mosaic males. No female mosaics were observed. However, the numbers are small and further tests must be made to determine whether polyspermy may not occur in binucleate eggs in *Habrobracon.* Two gynandromorphs with patroclinous male parts have been interpreted as being derived from binucleate polyspermic eggs (Whiting, 1931).

An individual was recently found by Magnhild Torvik Greb which can best be explained by assuming polyspermy in a binucleate egg. The individual is a female having left antenna aciform, right antenna type, left ocellar patch different in pigment from right and left gonapophysis shorter than right. Aciform, ac, causes reduction of diameter and often fusion of segments of approximately one-third of the antennæ at the distal end. It also reduces the size of the sensory gonapophyses, usually causing asymmetry. In this female, the left antenna was modified aciform, only the most distal segments being affected and these but very slightly—probably due to the fact that type tissue was also present. The genitalia were asymmetrical, the shortened gonapophysis being on the side with the aciform antenna. However, it cannot be definitely stated that the genital region is mosaic. This mosaic female appeared in a fraternity from a mother heterozygous for aciform which had been mated to an aciform male. Its mosaicism may be explained by assuming polyspermy and that the two oötids of a post-reduced binucleate egg (one carrying aciform, the other the normal allelomorph) were fertilized by sperm carrying the recessive aciform. Thus we could obtain a female, part aciform and part type.

Sex Ratios

X-radiation of mated females has been shown to affect sex ratio of their offspring as well as their fecundity (Whiting, 1929; Bostian, 1931). X-radiation increases rate of appearance of mosaics. It seemed possible that high temperature, which also increases the ratio of mosaics, might similarly affect sex ratio and fecundity.

TABLE II

Sex Ratio in Bisexual Fraternities

	Experiment	♀♀ Set	Progeny					
			♂♂	$\overset{\frown}{\text{♂♀}}$	♀♀	Per cent ♀♀	Sons per mother	Total per mother
A	Treated 5°–10° C.	61	1100	2	895	44.9	18	33
	Controls 30° C.	53	983	2	981	50.0	19	37
B	Treated −14°–10° C.	140	2469	6	1892	43.5	17	30
	Controls 30° C.	107	2226	4	1779	44.6	19	37
C	Treated 18°–20° C.	111	1101	0	691	38.6	10	16
	Controls 30° C.	78	1663	2	1167	41.2	21	36
D	Treated 35°–37° C.	76	1966	5	1171	37.3	26	41
	Controls 30° C.	65	2187	3	1562	41.6	34	58

A summary of offspring in bisexual fraternities is given in Table II. This summary brings out the fact that in all temperature experiments percentage of females is slightly lower among the offspring from treated than among the offspring from control females.

Fecundity

Fecundity of females, however, is more dependent upon temperature than is sex ratio. Again treated females always produced fewer offspring per mother than control females but all treatments did not have quite the same effect. The fecundity of the treated females in Experiment *A* was slightly decreased as compared with that of its control females. In Experiment *B*, there was a marked decrease in the number of offspring from treated as compared with offspring from controls. The very high temperature, Experiment *D*, reduced the fecundity by a little less than a third while the constant cold temperature decreased it by more than one-half.

Constant cold and extreme heat both had very significant effects on fecundity though they apparently acted in different ways. Cold acted by slowing down all the activities of the adult wasps thereby decreasing the rate of egg production. Cold also kept many of the eggs that were laid from hatching and killed many of the young larval wasps. Heat treatment apparently had little or no effect on rate of egg production. It affected the young, the consequences being especially noted in the pupal stage. There were many naked pupæ and many dead in the cocoons.

Anomalous Types

During the course of these experiments five mutant individuals were observed—each in a different fraternity. Four carried recurrent mutations to orange—a not infrequent mutation—from mothers heterozygous for ivory, Oo^i, an allelomorph of orange (Whiting, 1932, p. 297). These occurred in the controls. The fifth was at first mistaken for a gynandromorph with a female head and a male abdomen. However, the individual mated with a female and the trait, called gynoid, gy, was inherited as though due to a simple recessive factor. Gynoid males have female-like antennæ and abdominal sclerites. Homozygous gynoid females are indistinguishable from type. The first gynoid male, the mutant, appeared in the first of three vials of offspring from a heat-treated female (Experiment *D*, Table I).

Numerous other anomalous individuals were noted. Wasps with one large medial eye, " cyclops," were rather frequent among offspring from mothers treated with high temperature.

SUMMARY AND CONCLUSIONS

1. Short time exposure of heterozygous females to extreme cold did not significantly affect rate of production of male mosaics and gynandromorphs.

2. When such females were placed at a constant low temperature no mosaic offspring appeared.

3. Females kept at 35 to 37° C. produced a significantly greater number of such individuals than appeared in any other case.

4. Temperature treatment of mated females slightly affects the sex ratio of offspring, the most significant decrease in female ratio being observed at constant low or high temperatures.

5. Temperature treatment affected fecundity although short time exposure to cold had only a slight effect. Constant high temperature decreased fecundity by a little less than a third and constant low temperature lowered it to less than half that of the control wasps.

The writer wishes to render grateful acknowledgment to Professor P. W. Whiting, under whose direction the work was done, and to express his obligation to Professor R. T. Hance, who, as the head of the department, coöperated generously in supplying laboratory space and equipment. The grant to Dr. Whiting by the Committee on Effects of X-radiation on Living Organisms (National Research Council) made much essential technical assistance available.

LITERATURE CITED

Bostian, Neita C., 1931. Sex Ratios and Mutants from X-rayed Adult Females of Habrobracon. (Abstr.) *Anat. Rec.*, **51**: 121.

Goldschmidt, Richard, 1931. Die Sexuellen Zwischenstufen. *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere,* **23**: 437–455. Julius Springer, Berlin.

Greb, Raymond J., 1933. Effects of X-radiation on Production of Mosaic Males and on Sex Ratio in Habrobracon. *Am. Nat.*, **67**: 88.

Rösch, G. A., 1926. Über einen Weg, Zwitter der Honigbiene (Apis mellifica L.) im Experiment zu erzeugen. *Sitz.-Ber. d. Ges. f. Morph. u. Physiol. in München,* **37**: 71.

Rösch, G. A., 1928. Experimentelle Untersuchungen über die Enstehung von Zwittern bei der Honigbiene (*Apis mellifica* L.). *Verhand. der deutsch. Zool. Gesell.*, **32**: 219.

Whiting, Anna R., and Carey H. Bostian, 1931. The Effects of X-Radiation of Larvæ in Habrobracon. *Genetics,* **16**: 659.

Whiting, P. W., 1924. Some Anomalies in Habrobracon and their Bearing on Maturation, Fertilization, and Cleavage. *Anat. Rec.*, **29**: 146.

Whiting, P. W., 1929. X-rays and Parasitic Wasps. *Jour. Hered.*, **20**: 268.

Whiting, P. W., 1931. Diploid Male Parts in Gynandromorphs of Habrobracon. *Biol. Bull.*, **61**: 478.

Whiting, P. W., 1932. Modification of Traits in Mosaics from Binucleate Eggs of Habrobracon. *Biol. Bull.*, **63**: 296.

Whiting, P. W., and M. F. Stancati, 1931. A Gynandromorph of Habrobracon from a Post-reduced Binucleate Egg. *Biol. Bull.*, **61**: 481.

THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON MICROBRACON HEBETOR SAY (HYMENOPTERA: BRACONIDÆ) AND ITS HOST EPHESTIA KÜHNIELLA ZELLER (LEPIDOPTERA: PYRALIDÆ). I

NELLIE M. PAYNE

(*From the Zoological Laboratory, University of Pennsylvania*)

Introduction and Literature

A change in environment either physical, as temperature, light, or humidity, or biological, as the introduction of a new parasite, a predator, or a disease, may upset the equilibrium established between host and parasite. Certain conditions may favor the host; others, the parasite. Escherich and Miyajima (1911, 1912) described an interesting relation between the polyhedral disease in the nun moth, *Porthetria monacha* Linn., and weather changes. The latent form of the disease developed into the chronic, fatal form when nun moth larvae were exposed either to bright sunlight or to decrease in temperature. Field studies showed that outbreaks of the disease were correlated with sudden changes in weather. Růžička (1924, 1925) found that there was a periodicity in the outbreaks of the nun moth and that these outbreaks were associated with sun spot cycles. Weather cycles affected the parasites more than the hosts. If the spring temperature is high and the humidity low, the host is favored; if the spring temperature is low and the humidity high, the parasites. Both Tachinidae and fungi, parasitic on *Porthetria monacha* Linn., are favored by the same factors of high humidity and low temperature.

One of the most carefully studied cases of the differential action of environment on host and parasite is that of the "green bug" or grain aphis, *Toxoptera graminum* Rond., and its chief parasite, *Aphidius testaceipes* Cress. Webster and Phillips (1912) approached the problem of balance between the "green bug" and its parasite by determining the threshold for activity of each. The host was active and able to oviposit at a temperature lower than that required for the parasite. Warm weather was more favorable to the parasite than to the "green bug" itself. Headlee (1914) again attacked the problem of balance between host and parasite with the aid of special apparatus for controlling humidity and temperature.

From the data of Hunter (1909) on the number of aphids infested by a single parasite, from the results of Glenn (1909) on developmental threshold, and from the data of Headlee (1914) on the effect of temperature and humidity on host and parasite, Shelford (1926) came to the conclusion that it was impossible theoretically for the parasite to overtake the host. Shelford's method of determining a threshold of development was quite similar to that of Sanderson (1910) and Krogh (1914), but he used a theoretical α in place of the developmental zero of these authors. He also summed up developmental units instead of temperature, and concluded that since the parasite required more developmental units than the host, it could not exterminate the host.

The differential effect of environment on host and parasite has been worked out for the beet fly, *Pegomyia hyoscini* Panz., and its parasites; the gall midge, *Rhabdophaga heterobia* H. Lw., and its parasites; and the hawk moth pupa, *Protoparce quinquemaculatus* Haw., and its parasites.

According to the researches of Bremer (1928), and Blunk, Bremer, and Kaufmann (1928), cold weather retards the development of the parasites, *Opius fulvicollis* Thoms. and *Phygadeuon pegomyiae*, of the beet fly, *Pegomyia hyoscini* Panz., more than it does the host. Thus during a cool summer the beet fly gains on its parasites. In warm weather the parasites gain on it.

Barnes (1930) found that the parasites of the gall midge, *Rhabdophaga heterobia* H. Lw., were less cold-hardy than the midge itself. The percentage of survival of both *Dasyneura alopecuri* Reuter and *D. pyri* Bouché after exposure to low temperature was less than their host. This conclusion was based largely upon material collected in the field.

Hefley (1928) showed that as the relative humidity increased, the viability of the hawk moth pupa, *Protoparce quinquemaculatus* Haw., decreased. The viability of the parasite, *Winthemia quadripustulata* Fab., varied directly with increasing relative humidity. Above 73.4 per cent relative humidity emergence of the parasite was practically 100 per cent. In other words, a low relative humidity was favorable to the host; a high one, to the parasite.

Host-parasite balance may be affected not only by a physical factor of the environment, but also by a biotic factor. The effect of competition between parasites may be considered as the effect of a change of biotic environmental resistance on the balance between a single host and parasite. Important as is this problem to biological control work there is very little data upon it. Pemberton and Willard (1918) found that the total parasitic infestation of the fruit fly, *Ceratitis capitata* Wied., decreased when to the very efficient parasite, *Opius humulis* Silves., was added a less efficient parasite, *Diachasma tryoni* Cam. Larvæ of this parasite killed the larvæ of *Opius humilis* Silves. with their sharp mandibles. The killing of *Opius* by *Diachasma* was undesirable since *Opius* survived the winter better than *Diachasma.*

LITERATURE ON HOST-PARASITE BALANCE OF EPHESTIA KÜHNIELLA ZELLER AND MICROBRACON HEBETOR SAY

Efforts to evaluate *Microbracon* as a control measure for its host, *Ephestia,* have to a certain degree been attempts to determine the balance between this particular host and its parasite.

Buchwald and Berliner (1910) considered that *Microbracon hebetor* Say (*Habrobracon hebetor* Say) was a valuable aid in control of the Mediterranean flour moth. Back (1920) mentioned *Microbracon hebetor* Say and *Omorga frumentaria* Rond. as accessory controls for *Ephestia,* but thought that heat treatment or fumigation must be relied upon for effective combat.

Hase (1922, 1925) made an attempt to evaluate the parasitic efficiency of *Microbracon hebetor* Say (*Habrobracon brevicornis* Wesmael.) in controlling *Ephestia kühniella.* He let loose 100 *M. hebetor* adults and 500 *E. kühniella* larvæ in a space with a cubic content of 9,264 cubic meters with a surface of 387,348 square meters. He recovered 129 host larvæ and of these 70 were parasitized. Later he conducted similar experiments in which he found a parasitism of 50 to 60 per cent. From the concentration of *Microbracon hebetor* necessary to parasitize 50 per cent of the flour moth caterpillars he calculated that 20,000 female *M. hebetor* per 1,000 cubic meters would be needed for complete control. Hase did not reckon on any cumulative effects of several generations of parasites, but only on one. However, Muir (1913) has shown that a parasite may be overtaking a host when the host is apparently gaining.

METHODS AND MATERIALS USED IN THE PRESENT EXPERIMENTS

Two genetic strains of the Mediterranean flour moth, one of which developed much more rapidly than the other, were used in these experiments. Pure lines of the parasite, *Microbracon hebetor* Say, were not kept, but there was little variation between the reactions of different individuals to temperature. The females were almost pure yellow at 30° C. and almost black at 16.5° C. The stock was remarkably free from abnormal individuals. No twisted wings, white eyes, or stumpy antennæ were observed. Undersized adults were produced only when too many parasite larvæ fed on one host caterpillar, or when parasites fed on pink host larvæ.

Temperature studies on *Microbracon hebetor* Say were begun at the Biologische Reichsanstalt in Berlin-Dahlem and continued at the Marine Biological Laboratory at Woods Hole, Massachusetts, and at the University of Pennsylvania. The temperature equipment here described is that at the Anstalt in Dahlem. With the exception of the series incubators, the apparatus at Woods Hole and at the University of Pennsylvania was similar to that used at Dahlem.

The temperature control apparatus at Dahlem consisted of incubators in series, one at low temperature, the other at high, and also of individual incubators. There was no circulating air in them. For the insects in question the lack of circulating air is not a serious fault, but it would be for insects eating green food. The low temperature series of incubators was kept in a basement which itself was at low temperature. The compartments in the series incubator were kept at 16.5° C., 11.5° C., 9° C., 8° C., 5° C., 3.5° C., 2.2° C., and .9° C. The average deviation from the temperatures in question was ± .5° C. and the greatest deviation ± 1.0° C. Temperatures were read twice daily. The high temperature series was less accurate than the low. Gas was used as the source of heat. Temperatures of 38° C., 36.5° C., 32° C., 30° C., 28° C., 27° C., 25.4° C., 23.5° C., and 19° C. were maintained in the high series. The first three compartments on the high side and the first two on the low did not vary from the mean more than ± 1° C. The others could be used only for rough comparative studies to block out limits of duration of different stages. These limits could then be determined more accurately with individual incubators. In one series of experiments the compartment at 36.5° C. fell to 34° C., at which temperature it remained constant. Since the temperature remained constant at 34° C., opportunity was given to rear two generations of *Microbracon hebetor* and one generation of *Ephestia kühniella*. In all incubators, but in the series ones especially, it is important that the thermometer used for reading the temperature be at the same level as that at which insects are kept. The thermometers at the tops of the series compartments could not be used as fair indicators of the temperatures inside.

Individual incubators kept at 10° C., 16° C., 25° C., 27° C., and 30° C. were used. Two rooms, one kept at 18°–19° C., and the other at 15° C., were also available. Since it is much more difficult to maintain a constant temperature lower than the external environment than to maintain a temperature higher, the best results for constant low temperatures were obtained by placing an incubator in a room lower than the temperature desired. A room at 4° C. was used for the 10° C. and 16° C. incubators.

Host larvæ were reared in Petri dishes, in cylindrical jars, and in cardboard boxes. They were fed on rolled oats. In experiments to determine host-parasite balance, 10 adult parasites were placed with 100 host larvæ, eggs, or pupæ.

Thompson (1922) proposed a formula, later corrected by Chapman (1925), which could be used to calculate the number of generations required to overtake a host. A simple case was assumed, in which the parasite laid only one egg on each host attacked. Environmental factors were supposed to act equally favorably on both host and parasite.

The formula will be developed as follows:

If n = the original population of the host,
l = the sex ratio of the host,
h = the number of young produced by each female host,
Then, $lh = w$, the reproductive power of the host (reproductive potential).
If p = the original population of the parasite,
f = the sex ratio of the parasite,
s = the number of young produced by each female parasite,
Then, $fs = z$, the net reproductive power of the parasite (reproductive potential).

Then, nw = the number of hosts after the first generation,
pz = number of parasites after the first generation,
$nw - pz$ = number of surviving hosts after the first generation.

k = number of parasite generations to equal host population.

The number of generations, k, required for the number of parasites to equal the number of hosts is found when

$$(nw^k \cdots) - pz^k = 0,$$

or the number of parasites is equal to that of the host. k may be found by using the mathematical transformation

$$\theta = \frac{w}{z}, \qquad \beta = \frac{zp}{n(z - w) - zp} \qquad \text{and} \qquad k = \frac{\text{Log } \theta}{\text{Log } \beta}.$$

If w is greater than z or in other words, if the reproductive power of the host is greater than that of the parasite, p can never equal h, and there is no theoretical possibility that the parasite can exterminate the host.

TABLE I

Development of Ephestia kühniella

Temperature	Time required for fast strain	Time required for slow strain	Number of generations per annum of fast strain	Number of generations per annum of slow strain
° C.	*days*	*days*		
32	35– 37	62– 65	10.4–	5.8
30	41– 43	66– 68	8.9–	5.5
27	43– 45	69– 72	8.5	5.3
25	50– 53	79– 81	7.3	4.6–
20	63– 65	99–103	5.8	3.7–
15	85– 90	135–157	4.3	2.6–
10	140–175	205–243	2.6	1.08
8	cannot develop	250–270	—	1.04

If $w = z$, or in other words, the reproductive powers of the host and parasite are equal, then

$$k = \frac{h}{p},$$

or the number of generations required by the parasite to equal the numbers of the host is equal to the quotient of the original number of hosts, divided by the original number of parasites.

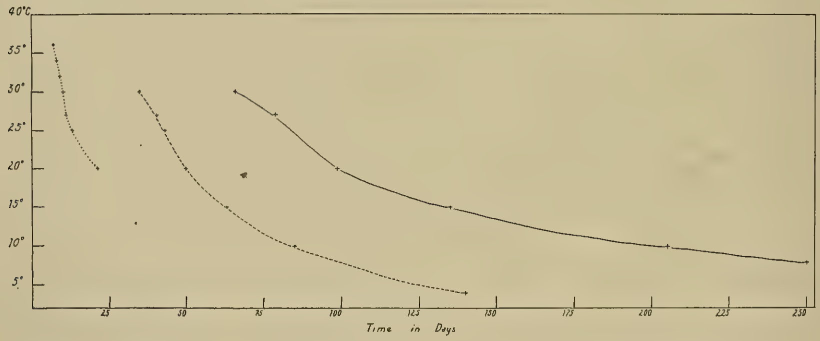


FIG. 1. Duration of development of host and parasite from the time the egg is laid until the emergence of the adult, plotted against temperature.

Dotted line, parasite (*Microbracon hebetor*). Broken line, fast strain of host (*Ephestia kühniella*). Solid line, slow strain of host (*Ephestia kühniella*).

However, *Microbracon* may lay as many as 22 eggs on a single host. The number of eggs laid on a caterpillar stung by the host varies from 0–22, with a mean value of 10. If a parasite lays 10 eggs on one host, it does not affect the host population as much as if it laid one egg on each of 10 hosts. The number of generations required

TABLE II

Development and Length of Life of Microbracon hebetor

Temperature	Time required for development	Number of generations per annum	Median life length of female
° C.	*days*		*days*
36	7	52 –	3
34	8	45.6	6
32	9	40.5	9
30	10–11	36.5	13
27	11–12	33.2	20
25	13–14	28.7	23
20	19–20	19.2	28
16.5	—	—	55
10	—	—	90

for the parasite to overtake the host when the parasite lays more than one egg per host may be expressed by the following equation,

$$k = \frac{\log \theta}{\log \beta} = \frac{\log \dfrac{w}{\dfrac{z}{m}}}{\log \dfrac{\dfrac{zp}{m}}{n\left(\dfrac{z}{m} - w\right) - \dfrac{zp}{m}}}$$

with the additional symbol m, which represents the number of eggs laid per host by a parasite. For the expression z/m, a new symbol, ϵ, may be used to designate parasitic efficiency. Then the equation reads,

$$k = \frac{\log \dfrac{w}{\epsilon}}{\log \dfrac{\epsilon p}{n(\epsilon - w) - \epsilon p}}.$$

In order to determine the effect of temperature upon host-parasite balance, both strains of the host and the one strain of parasite were reared separately. Then host and parasite were put together.

RESULTS OBTAINED

Effect of Temperature upon Life Cycles of Host and Parasite

The slow strain of host can survive and complete its developmental cycle at temperatures from 34.4° C. to 8° C. inclusive. The larva of the slow strain can live at 36° C., but cannot pupate. The fast strain of host cannot complete its developmental cycle at temperatures below 10° C. The time required from deposition of the egg to emergence of the imago is shown in Table I. As will be seen by the table, the potential number of generations per annum of the two strains differs widely at each temperature. Although the durations of the life cycles of the slow and of the fast strain differ markedly, yet the effect of temperature on the rate of development of the two strains is approximately the same. The similarity of the curves of the two strains made when duration of development is plotted against temperature is shown in Fig. 1.

TABLE III

Reproductive Potential of Ephestia kühniella

Temperature	Eggs laid per female	Sex ratio	* Fertility of fertilized females	Reproductive potential
° C.				
32	181	54	.70	68.618
30	204	54	.70	77.108
27	225	54	.70	85.05
25	200	54	.70	75.60
20	177	54	.65	62.127
15	156	54	.50	42.12
10	67	54	.10	3.618
8	55	54	.10	2.97

* Fertility expressed as percentage of females laying eggs.

TABLE IV

Reproductive Potential of Microbracon hebetor

Temperature	Eggs laid	Sex ratio	Reproductive potential	Parasitic efficiency
° C.				
36	90	.75	67.50	6.75
34	105	.75	78.75	7.875
32	111	.75	83.25	8.325
30	114	.75	87.50	8.75
27	120	.75	90	9
25	113	.75	85	8.5
20	87	.75	65.25	6.52
15	56	.50	28	2.8
10	—	—	—	—

The parasite, *Microbracon*, can live and complete its life cycle at temperatures from 36° C. to 14.5° C. The adult could survive for long periods at lower temperatures, but could not lay eggs. Length of time from egg to emergence of adult, and the median life length of the adult female as it varies with temperature, are shown in Table II. The curve produced when temperature is plotted against duration of development is shown in Fig. 1.

Not only is the duration of development affected by temperature, but the reproductive ability also. The biotic potential at any given temperature may be calculated by multiplying the average number of eggs laid per female, by the decimal representing the percentage of the population which is female, and this product by the percentage of females which lay eggs. Fertility is used in the statistical rather than in the physiological sense. The biotic potential of *Ephestia*, the host, is shown in Table III; that of *Microbracon*, the parasite, in Table IV. The maximum biotic potential of both host and parasite is reached at 27° C., as shown in Fig. 2.

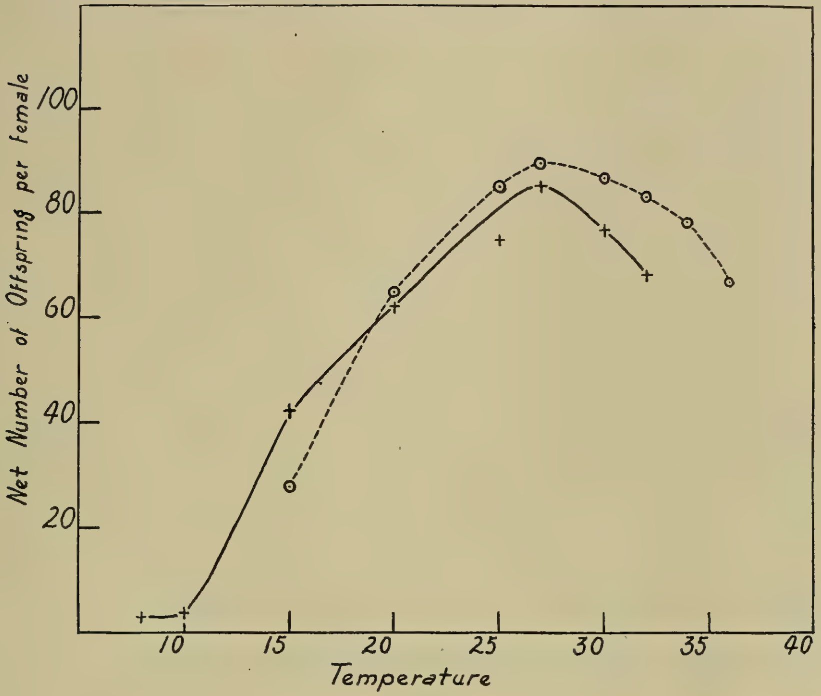
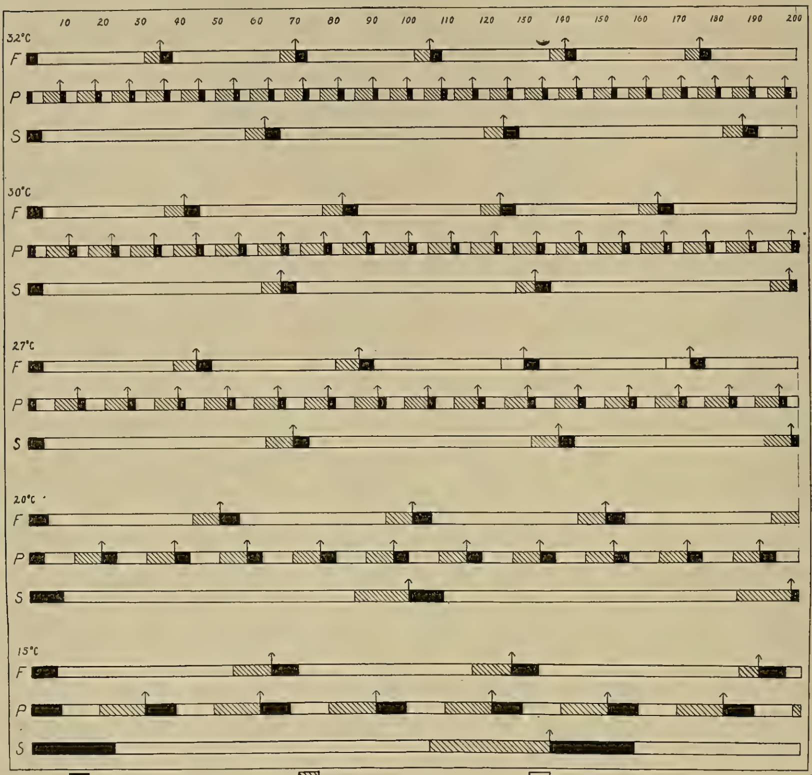


FIG. 2. Reproductive potential of host and parasite at the different temperatures studied. Solid line, *Ephestia kühniella* (host). Broken line, *Microbracon hebetor* (parasite).

As seen from Table V, or better from Fig. 3, the life cycle of the parasite is characterized by a comparatively short larval stage and a relatively long pupal stage. The host has a relatively long feeding stage and rather short resting stages, namely, the pupa and the egg.



■ = egg. ▨ = pupa. ☐ = larva.

F = fast strain of host. S = slow strain of host. P = parasite. ↑ = emergence.

FIG. 3. Length of egg, larval, and pupal stages, and the time of emergence of two strains of host (*Ephestia kühniella*) and one of parasite (*Microbracon hebetor*).

Since the life cycles of the host and the parasite are not synchronous, the possibility of the parasite catching up with the host had to be determined under two conditions, (1) when the parasite emerged during a period of the host on which it could feed, and (2) when the parasite emerged during a period of the host on which it could not feed. The parasite can feed on the larval stage only of the host.

Host-parasite Balance if the Parasite is Set on the Larval Stage of the Host

The ability of the parasite to control the host depends upon its relative reproductive rate and also upon its relative life length in comparison with those of the host. The initial number of hosts in the experiments to be described was 100; the initial number of parasites, 10. Results of some of the experiments in host-parasite balance are shown in Table VI.

TABLE V

Time Required for Development

Temperature	Ephestia kühniella								Microbracon hebetor			
	Slow strain				Fast strain				Egg	Larva	Pupa	Total
	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total				
° C.	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
36	—	—	—	—	—	—	—	—	1	3	3	7
34	—	—	—	—	—	—	—	—	1	3	4	8
32	4	53	5	62	3	28	4	35	1.5	3	4.5	9
30	4	57	5	66	4	32	5	41	2	3	5	10
27	4	58	7	69	4	33	6	43	2	3	6	11
25	5	64	10	79	5	38	7	50	2	5	6	13
20	9	76	14	99	7	46	10	63	4	8	7	19
15	22	82	31	135	18	53	14	85	8	10	12	30
10	34	122	49	205	27	75	38	140	—	—	—	—
8	43	143	64	250	—	—	—	—	—	—	—	—

At temperatures from 32° C. to 20° C. inclusive the parasite was able to overtake the host. As shown in Table VI, at the close of the first generation the numbers of the host were not always appreciably diminished. However, by the end of the second generation the host was annihilated. The possibility of the parasite overtaking the host is not only a theoretical possibility, but also a practical fact that has to be taken into consideration when rearing hosts and parasites. A large extra supply of host larvæ must be kept on hand in order to keep the parasite culture going, since the parasites in killing off their hosts also destroy their own food supply.

Under the conditions of the experiment the parasite required 18 days to overtake the host at 32° C., 20 days at 30° C., 22 days at 27° C., 26 days at 25° C., and 39 days at 20° C.

At 15° C. the parasite was able sometimes to overtake the host, sometimes not. The possibility depended upon whether or not the parasite was able to complete two generations on the host larvæ. There was more variation both in generation length of the parasite and in the length of the host larval period at extreme temperatures than at medial temperatures. If the slow strain of host larva required the minimum time to develop, namely 82 days, and the parasite the minimum time, the parasite would be able to catch up with the host. If the parasite required the maximum period for development, namely 40 days, and the slow strain of host 82 days, the parasite would not be able to produce two generations on the host larva nor to overtake

TABLE VI

Host-parasite Balance at the Different Temperatures Used

Temperature	Larval period of host		Length of parasite life cycle	Initial number of parasites	Initial number of hosts	Hosts killed by parasite. 1st generation	Hosts remaining	Parasite potential by second generation	Hosts remaining
	Slow strain	Fast strain							
° C.	*days*	*days*	*days*						
32	53	28	9	10	100	83.20	17.80	6952	—
30	57	32	10	10	100	87.50	12.50	76542.5	—
27	58	33	11	10	100	90	10	81000	—
25	64	38	13	10	100	85	15	72250	—
20	76	46	19	10	100	76	24	57760	—
15	82–90	53–62	30–40	10	100	28	72	*784.00	—

* If host larva in fast strain requires only minimum time the parasite can never catch up with it.

TABLE VII

Comparative Life Length of Adult Parasite and Non-feeding Stages of the Host

Temperature	Life length of adult parasite	Duration of egg stage of host		Duration of first larval stadium		Duration of egg + pupa of host		Duration of pupa + egg + 1st instar larva of host		Duration of egg + 1st instar	
		Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain
° C.	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
32	9	4	3	8	4	9	7	17	11	12	7
30	13	4	4	8	5	9	9	17	14	12	9
27	20	4	4	9	6	11	10	20	16	13	10
25	23	5	5	11	7.5	15	12	26	19.5	16	12.5
20	28	9	7	12	9	23	17	35	26	21	16
15	53	22	18	18	12	53	32	71	44	40	36
10	90	34	27	—	—	—	—	—	—	—	—

the host, when allowance is made for the length of the first instar of the host larva on which the parasite cannot feed.

If the fast strain of host requires the minimum period in which to develop and the parasite also requires the minimum, the parasite can produce only one generation on the host larva, and cannot overtake the host. In order for even the most rapidly developing parasite to reproduce twice on a single generation of host larva, the larval period of the host must be at least 60 days long. Even on the slowest developing host larvæ of the fast strain, the parasites are unable to produce more than one generation, if they require more than 31 days for a life cycle. The chances that 10 parasites will be able to overtake 100 fast strain hosts when set on fast strain larvæ at the beginning of the larval period are about 1 : 16, if there is no pre-imaginal mortality among the parasites.

However, at 15° C. there is often pre-imaginal mortality among the parasites. A drop of water may condense on the dish in which the culture is kept and the parasite may drown in it. Drowning of young parasites may be set down to poor technique. Even if the dishes are kept free from water of condensation, young parasites die. At 15° C. the adult parasite is very clumsy. The female may miss the ganglion when she stings the host caterpillar, thus failing to paralyze the host larva and consequently failing to provide food for the young parasite. Eggs are often laid some distance away from the host larva. At 15° C. the female parasite may lay eggs so far away from the host larva that the parasite larvæ die of starvation before they are able to find their food. Not only is the adult clumsier, but also the young larva seems to be more poorly coördinated than at high temperature. If as many as three larvæ of the first generation of the parasite die, the parasite is unable to overtake the fast strain of host.

Below 15° C. there is no possibility of the parasite catching up with the host, since the parasite cannot complete its life cycle, although the adult can survive temperatures of 0° to + 4° C. for a period of several months. It is also of importance to realize that only the females which have been reared at low temperatures are capable of laying eggs at 14.5° C. and 15° C.

At temperatures above 32° C. the parasite has a decided advantage over the host. The maximum temperature at which the host can complete its life cycle is 34.4° C., but the parasite is able to complete its life cycle at a temperature as high as 36° C. The larval stage of the host, however, can survive 36° C., and thus provide food for the parasite.

Host-parasite Balance if the Parasite is Set upon a Host Stage on which it Cannot Feed

Since the life cycles of the host and parasite are not synchronous, parasites may emerge at a time when there is no food for them. They cannot feed on the egg, the pupa, or the first larval stadium. If adult parasites emerge at the beginning of the pupal period of the host, they must wait through this period, through the emergence and sexual maturity of the adult host, through the egg stage of the adult, and in addition through the first larval stadium. Since the adult female of *Ephestia* generally lays eggs on the day in which she emerges, the pre-oviposition period of the host is calculated in with the egg stage. If the parasite emerges at the beginning of the egg stage it must survive both this stage and the first larval stadium. Table III shows the median life length of the adult parasite as compared with the host stages upon which it cannot feed.

If the adult parasite is set on the slow strain of host at the beginning of the pupal stage it cannot survive at 32° C., 30° C., 25° C., 20° C., or 15° C. The parasite can survive at 27° C., at which temperature the biotic potential of both host and parasite reaches its maximum. Not only can the parasite survive, but it can also overtake the host under certain conditions. If the parasite is able to lay viable eggs on the last day of life the parasite can survive. For the strain of *Microbracon* used, the number of eggs laid each day was practically constant throughout life. Therefore, if the net number of offspring for each parasite during its entire life was 90, the number of offspring left by a parasite the last day of its life would be one-twentieth of 90, or 4.5. For 10 *Microbracon* the number would be 45, which offspring would be capable of exterminating 4.5 hosts. At the end of the first generation of parasites, the host numbers would have decreased but slightly. However, these 45 individuals produced would have their full reproductive power, and could produce 4050 offspring, capable of parasitizing 405 hosts. In the meantime the 100 original hosts would have increased their numbers to 7715, when the emerging adults laid eggs. At the end of the second generation of parasites the number of hosts would be 7310. The third generation of parasites would consist of 364,500 individuals capable of parasitizing 36,450 hosts, or several times the hosts remaining.

If the 10 original parasites produce only 10 offspring, or one per parasite, the parasites will yet be able to overcome the host by the end of the third parasite generation.

If the adults of *Microbracon hebetor* are set on the fast strain of host at the beginning of the pupal period they will be able to survive at all temperatures from 27° C. to 15° C. inclusive, and to overtake the host at 27° C., 25° C., and 20° C., but not at 15° C. At 15° C., even when there is a stage of the host present upon which the parasite can feed, the reproductive power of the parasite is insufficient to overtake the fast strain of host. When the effective reproductive power is lowered by a period of starvation, the parasite is unable to overtake the host.

If the adult *Microbracon* are set on either strain of host at the beginning of the egg stage, the *Microbracon* are able both to survive and to overtake the host, except at 15° C.

When the survival of the host depends upon its ability to starve out the parasite, the slow strain has the advantage, since the pupal and the egg periods are longer in it. When survival of the host depends upon a rapid enough development in the larva to prevent two generations of parasites from developing, the fast strain of host has the advantage. Since the larval period of either host strain is longer than the combined duration of the other periods in the life cycle (see Fig. 3), the fast strain has the advantage of the slow one, considering the life cycle as a whole.

Humidity as Affecting Host-parasite Balance

The adults of the parasite, *Microbracon hebetor* Say, are extraordinarily resistant to low relative humidity. They can be kept over anhydrous $CaCl_2$ throughout life with no apparent harm. The eggs, spun larvæ, and pupæ are also resistant to low relative humidity. The larval stage, which is of comparatively short duration as compared with the other stages in the life cycle, is the only stage which can be injured easily by an atmosphere of low relative humidity. Although the larvæ are not as resistant to drying as are the other stages, even they are injured and may die in a saturated atmosphere. At 15° C. young larvæ frequently die if the relative humidity is not kept low by an appropriate salt. The larvæ are sensitive to relative rather than to absolute humidity, since the absolute humidity at saturation at 15° C. is less than the absolute humidity with a 50 per cent saturation at 20° C., for example.

The host, *Ephestia kühniella* Zeller, is sensitive to low relative humidity. Low humidity is associated with increased sterility in the adults and with production of large numbers of crippled and deformed individuals. Low humidity during the pupal stage especially is reflected by appearance of deformities in the adult stage. The larvæ are able to withstand but little drying. They prefer damp food to dry and can with difficulty gain foothold on sound, dry products.

Once, however, the larvæ infest a given material, they raise its moisture content and thus make the environment favorable for more of their species.

The host, *Ephestia kühniella*, cannot live and reproduce under the low relative humidities that the parasite can; the parasite cannot live in the damp atmosphere that favors the host. At intermediate humidities both can live and reproduce.

Factors Other than Physical which Influence Host-parasite Balance

The color of the host larvæ, the presence of "*Microbracon* immune" strains, and the opposite reactions of host and parasite to light also influence host-parasite balance.

There are two colors of full-grown host larvæ, a pinkish and a white or greenish. The pink larvæ are unable to support as large a population of *Microbracon* as are the white ones. In a mixed group of pink and white host larvæ the female parasite stings the white larvæ before she does the pink ones. The chemical nature of the pigment difference between the two strains of larvæ is not as yet known. Color of the larvæ is, however, an inheritable character. Complicating factors in determining the genetics of color are changes of color with advancing age of the larvæ, and the effect of certain environmental factors on color.

In a given population of host larvæ exposed to parasitic attack some may be immediately stung by the parasites. Others may be oviposited upon when the choice of host larvæ has become very limited. Finally, there exist certain host larvæ which are apparently "*Microbracon* immune." These are not stung by the parasitic wasps even if there are no other larvæ available.

The degree of *Microbracon* immunity of *Ephestia* larvæ was determined by allowing 10 *Microbracon* access to 100 *Ephestia*. At the end of the first day those *Ephestia* which were not stung were exposed to fresh *Microbracon*. If they were not stung by the second day they were exposed a third and so on, until the only larvæ remaining were those which the *Microbracon* did not attack. About one larva in 3000 appeared to be absolutely immune to the strain of *Microbracon hebetor* used in the experiments.

The *Microbracon* adults are strongly positively phototropic and the *Ephestia* larvæ negatively phototropic. The adult parasite generally flies directly toward the source of light when it emerges, even if host larvæ are present. Host larvæ avoid light.

When mixed populations of host larvæ and parasite adults were kept in cylindrical jars or Petri dishes, the parasite was able to exterminate the host. When, however, the cultures were placed in square cardboard boxes the hosts were able to survive. They would spin up in dark corners. Once covered with a cocoon they were attacked with difficulty by the *Microbracon* adults. In the square, cornered containers the better protection of the larvæ apparently was not due alone to their light reactions, but also to their strong positive thigmotropism.

Presence of disease in the host larvæ may affect balance between the insect parasite and its host. If a host larva becomes infected with a fatal disease, the parasites upon it die with it. In the early stages of both sporozoan and bacterial infection the infected larvæ move about more slowly than do normal larvæ and are more subject to parasitic attack. The elimination of the parasites feeding on diseased larvæ may in some cases enable the host population to survive, even when it loses some of its members through disease. In general it may be said that if a parasitized larva becomes diseased, the host-parasite balance is tipped in favor of the host; if a non-parasitized larva becomes diseased the host-parasite balance is tipped in favor of the parasite. The above conclusion holds only if the disease is a fatal one. If the disease is non-fatal, it influences host-parasite balance if it makes the host larva a less nourishing food for the parasite or if it delays maturity of the host. In the former case the host would be favored; in the latter, the parasite.

Discussion

When disease is not present and "*Microbracon* immune" host larvæ do not form a significant part of the host population, the Thompson formula as modified by Chapman predicts the possibility of a given parasite population exterminating a host population. In the original use of this formula, the assumption was made that the life cycles of host and parasite were synchronous. If these cycles are not synchronous, as is the case with *Ephestia* and its parasite, *Microbracon*, results calculated from the formula agree fairly well with those obtained from experiment, if account is taken of the decreased reproductive ability of the parasite, when it emerges during a stage of the host upon which it cannot feed.

Summary

1. Two strains of the Mediterranean flour moth, one of which develops much faster than the other at a given temperature, were used in the experiments reported in this paper. Only one strain of the parasite, *Microbracon hebetor* Say, was bred.

2. If the parasite emerges during the larval stage of the host and is able to complete two generations during this stage it can exterminate the host.

3. If the parasite emerges at the beginning of the pupal period of the host it can exterminate the slow strain of host only at 27° C., but it can overtake the fast strain at temperatures from 27° C. to 20° C. inclusive. If the adult parasite emerges at the beginning of the egg stage it can exterminate either strain of host except at 15° C. or below.

4. In general high temperatures (above 32° C.) favor the host; low temperatures (15° C. and below), the parasite.

5. High relative humidity favors the host; low relative humidity, the parasite.

6. Conditions other than physical, such as disease incidence and the webbing habits of the flour moth caterpillar, also affect the host-parasite balance.

LITERATURE CITED

BACK, E. A., 1920. Insect Control in Flour Mills. *U. S. Dept. Agri. Bull.*, 872. 40 pp.

BARNES, H. F., 1930. On Some Factors Governing the Emergence of Gall Midges: Cecidomyidæ: Diptera. *Proc. Zool. Soc. London*, 1930: 381–393.

BLUNK, H., H. BREMER, UND KAUFMANN, 1928. Untersuchungen zur Lebensgeschichte und Bekämpfung der Rübenfliege (*Pegomyia hyoscini* Panz.). *Arb. Biol. Reichsanstalt f. Land. u. Forstwirtsch.*, **16**: 423.

BREMER, H., 1928. Geschichte des Rübenfliegenbefalles in nördlichen Vorpommern und auf Rügen im Jahre 1925. *Arb. Biol. Reichsanstalt f. Land. u. Forstwirtsch.*, **16**: 448.

BUCHWALD, J., AND E. BERLINER, 1910. Habrobracon hebetor Say. Ein Bundesgenose im Kampf gegen die Mehlmotte. *Zeitschr. f. das ges. Getreidewesen*, **2**: 1.

CHAPMAN, R. N., 1925. Animal Ecology with Special Reference to Insects. Burgess-Brook Inc., Minneapolis. 370 pp. See pp. 158–161.

ESCHERICH, K., AND M. MIYAJIMA, 1911. Studien über die Wipfelkrankheit der Nonne. *Naturw. Zeitschr. Forst. Landw.*, **9**: 381.

ESCHERICH, K., AND M. MIYAJIMA, 1912. Studien über die Wipfelkrankheit der Nonne. *Biol. Zentralbl.*, **32**: 111.

GLENN, P. A., 1909. The Influence of Climate upon the Green Bug and its Parasites. *Kansas Univ. Sci. Bull.*, **9**: 165.

HASE, A., 1922. Biologie der Schlupfwespe, *Habrobracon brevicornis* Wesmael (Braconidae) zugleich ein Beitrag zur Frage der biologischen Bekämpfung von Schädinsekten. *Arb. Biol. Reichsanstalt f. Land. u. Forstwirtsch.*, **11**: 95.

HASE, A., 1925. Weitere Versuche zur Frage der biologischen Bekämpfung von Mehlmotten mit Helfe von Schlupfwespen. *Arb. Biol. Reichsanstalt f. Land. u. Forstwirtsch.*, **19**: 163.

HEADLEE, T. J., 1914. Some Data on the Effect of Temperature and Moisture on the Rate of Insect Metabolism. *Jour. Econ. Ent.*, **7**: 413.

HEFLEY, H. M., 1928. Differential Effects of Constant Humidities on Protoparce quinquemaculatus Haworth, and its Parasite, Winthemia quadripustulata Fabricius. *Jour. Econ. Ent.*, **21**: 213.

EDITOR'S NOTE:

Paragraph 4 of the summary of the article by Nellie M. Payne on p. 204 should read as follows:

"In general high temperatures (above 32° C.) favor the parasite, low temperatures (15° C. and below) the host."

HUNTER, S. J., 1909. The Green Bug and its Enemies. *Kansas Univ. Bull.*, **9**: 1.

KROGH, A., 1914. On the Rate of Development and CO_2 Production of Chrysalides of Tenebrio molitor at Different Temperatures. *Zeitschr. allgem. Physiol.*, **16**: 178.

MUIR, F., 1913. Presidential Address. *Proc. Hawaiian Ent. Soc.*, **3**: 28.

PEMBERTON, C. E., AND H. F. WILLARD, 1918. Interrelations of Fruit-Fly Parasites in Hawaii. *Jour. Agr. Res.*, **12**: 285.

RŮŽIČKA, J., 1924. Die neuesten Erfahrungen über die Nonne in Böhmen. *Centralbl. f. das ges. Forstw.*, **50**: 33–67; 313–335.

RŮŽIČKA, J., 1925. Einige Bemerkungen über die Nonnenbekämpfung auf biologischen Wege. *Fortwiss. Zentralbl.*, **47**: 537.

SANDERSON, E. D., 1910. The Relation of Temperature to the Growth of Insects. *Jour. Econ. Ent.*, **3**: 113.

SHELFORD, V. E., 1926. The Relation of Abundance of Parasites to Weather Conditions. *Jour. Econ. Ent.*, **19**: 283.

THOMPSON, W. R., 1922. Théorie de l'action des parasites entomophages. Accroissment de la proportion d'hôtes parasités dans le parasitisme cyclique. *Compt. Rend. Acad. Sci.* [Paris], **175**: 65.

WEBSTER, F. M., AND W. J. PHILLIPS, 1912. The Spring Grain aphis or "green bug." *Bull. Bur. Ent. U. S. Dept. Agr.*, **110**. 153 pp.

STUDIES OF THE MITOTIC FIGURE

III. CHÆTOPTERUS: CENTRAL BODY STRUCTURE AT METAPHASE, FIRST CLEAVAGE, AFTER USING DILUTED FIXATIVES.

HENRY J. FRY

(From Washington Square College, New York University, and the Marine Biological Laboratory, Woods Hole)

I. PURPOSE OF THE STUDY

A previous investigation of central bodies in the eggs of *Chætopterus pergamentaceus*, at metaphase, first cleavage, described the effects upon their structure of using various modifications of Boveri's picro-acetic reagent. It was found that central body configuration is closely related to the coarseness and shape of the astral rays, and that centrioles are demonstrated only when rays are very coarse and undulating (almost straight); this occurs only if the reagent is diluted with about 90 parts of water (Fry, 1932). Since such behavior of central bodies has not been reported elsewhere, and since those of *Chætopterus* have heretofore been regarded as typical centrioles, giving genetic continuity to successive division figures (Mead, 1898 and Wilson, 1930), it was thought desirable to determine whether or not the results produced by modifying a picro-acetic mixture hold true when diverse reagents are employed.[1]

II. METHODS

For the most part, the methods of the present investigation were the same as those of the earlier study. The eggs used were taken from many females, obtained at different times in the season during the past four summers, at the Marine Biological Laboratory at Woods Hole; they were handled as previously described. As before, eggs of the various sets were left in the reagents from 6 to 15 hours (it was previously shown that these differences do not affect astral types when Boveri's picro-acetic reagent is used, and it is assumed that the same

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[1] This study was completed by the aid of a grant from the National Research Council.

situation probably holds true in the case of other reagents). Eggs were again sectioned at a thickness of 5 μ and stained with Heidenhain's hæmatoxylin, every effort being made to destain them all to the same "medium" extent, in order that no variations in central body structure would be introduced by differences in depth of stain. As before, study was confined to the mid-sections of the larger aster of metaphase first-cleavage figures. Also the same method was used to secure a random sample of the various metaphase astral types which may be present in any egg-set, and thus to avoid an unconscious selection of any class.[2] Cells were studied with the same optical set-up and analyzed with reference to the same list of points (Fry, 1932, pp. 150–152 and 154–155).

The major data of the present study are reported in Table I (pp. 211–215) and include: first, the reagents employed; second, the structure of the central body and rays (astral type) demonstrated in each case; and third, the accompanying cytoplasmic configuration. Additional tables present various analyses of the data.

The 116 formulæ used fall into several categories: (*a*) most of the standard fixing fluids; (*b*) various original reagents; (*c*) the components of the different fluids used separately (in each case the amount dissolved is that usually called for when that component is used in standard reagents); and (*d*) dilutions of various members of the preceding groups.[3] Formulæ are given in Table I only when they are not found in Lee's *Microtomist's Vade Mecum*, ninth edition; page references are given for those found in that work.

The usual method of writing formulæ makes it impossible to appreciate at a glance the relative amounts of the components present in different reagents, because the items are called for in such terms as the following: "4 cc. of a 2 per cent solution of osmic acid," "corrosive sublimate to saturation," "3 drops of acetic acid," "99 cc. of a saturated solution of picric acid diluted with two parts of water," "1 gram of potassium dichromate," and "100 cc. of 60 per cent alcohol." In the present study, therefore, in addition to reporting the formulæ in the usual manner there is also reported in each case the percentage by weight of each component, as well as the total percentage by weight of all the solutes. This permits ready comparison of the similarities and differences between the various formulæ. The figures given for the percentages by weight are only approximately correct, because, regardless of the care with which the reagents are made up and the weights of their components calculated, small quantities of sea water are inevitably added to the reagent when the eggs are added, and the percentages of the components are thus modified to an unknown and uncontrolled degree.[4]

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[2] As previously reported (pp. 164–165), the exact manner in which eggs happen to be added to the reagent in the vial may involve the production of several astral types, as a result of uncontrolled dilution effects, occurring within the first second, when fixation takes place, and differing in various parts of the vial, depending upon how the egg suspension happens to mix with the fixative.

[3] The results obtained in the earlier investigation with Boveri's picro-acetic reagent, both full-strength and diluted, and with the two components used separately, are included here in order that the data may be compared with those of the present study.

Reagents made of the same components are arranged in groups in so far as that is possible. This permits ready analysis of such relations as the following: Is the presence of a certain component favorable to the demonstration of a given astral type, regardless of its amount or the presence of other components? If not, is the presence of that component, provided it occurs in certain amounts, favorable to showing that astral type and what is the effect of other components in various amounts? Is the total amount of solutes present in each reagent a significant factor?

The abbreviations used for the components are as follows:

Acetic acid	*Act*	Osmic acid	*Osm*
Alcohol, absolute	*Alc*	Picric acid	*Pic*
Chromic acid	*Chr*	Platinic chloride	*Plt*
Chloroform	*Clf*	Potassium dichromate	*Pot*
Ether	*Eth*	Sodium sulphate	*Sod*
Formaldehyde	*For*	Sublimate, corrosive	*Sub*
Nitric acid	*Nit*	Sulphuric acid	*Sul*
		Urea	*Ure*

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[4] Calculations of the percentages by weight were made as follows: (1) in the case of solids like chromic acid, the usual practice in preparing a 1 per cent solution is to add 1 gram to 100 cc. of water. While this is actually a 0.999 per cent solution by weight, it is here regarded as a 1 per cent solution. (2) Two solids, picric acid and corrosive sublimate, are commonly used in the form of saturated solutions: 1.22 grams of picric acid dissolve in 100 cc. of water at 20° C., and 6.5 grams of corrosive sublimate under the same conditions. In both cases the possible effects upon the amounts dissolved of relatively slight changes in room temperature and of the presence of other components were ignored, except in the case of the picro-sulphuric mixtures (formulæ 33 and 34), in which the sulphuric acid precipitates large amounts of the picric acid. (3) The percentage by weight of the fluids, glacial acetic acid, absolute alcohol, and ether, is determined by multiplying the number of cc. used by the specific gravity. (4) Chloroform is used as a saturated solution: 0.62 gram dissolves in 100 cc. of water at 20°. (5) The fluids which contain water—formaldehyde, nitric acid, and sulphuric acid—are treated as follows: (*a*) formaldehyde is assumed to be at 38 per cent strength in the average laboratory bottle (in contrast to the 40 per cent strength of a perfectly fresh solution), hence each cc. of fluid (sp. grav. 0.91) contains 0.34 gram of formaldehyde and 0.56 gram of water; (*b*) each cc. of nitric acid (70 per cent strength, sp. grav. 1.42) contains 0.99 gram of nitric acid and 0.42 gram of water; (*c*) each cc. of sulphuric acid (95 per cent strength, sp. grav. 1.84) contains 1.74 grams of sulphuric acid and 0.09 gram of water.

EXPLANATION OF PLATE I

Each figure was drawn from a cell typical of its class. A little less than half of the cell is shown, enlarged 1000×. The symbols are those used in the tables.

Fig.	Reagent	Astral Type			Cytoplasmic Type			
		Central Body	Rays	Symbol	Peripheral Bodies	Symbol	General Cytoplasm	Symbol
1	Boveri's picro-acetic, 10% strength (formula 41)	Centriole with slightly demarked centrosome	Very coarse, undulating	5 *A*	Unstained, with few exceptions	*Un**	Rough	*Ro*
2	Acetic acid, 0.5% sol. (formula 3)	Slightly demarked centrosome	Medium coarse, undulating	4 *B*	Absent	*Ab*	Rough	*Ro*
2a	Chloroform-acetic (formula 72)	Ascaris-like central body	Medium coarse, undulating	4* *B*				
3	Kahle's formol-alcohol-acetic (formula 12)	Undemarked centrosome, dense	Delicate, undulating	3 *C*	Unstained	*Un*	Intermediate	*In*
4	Boveri's picro-acetic (formula 37)	Undemarked centrosome, even	Medium coarse, undulating	2 *B*	Stained	*St*	Intermediate	*In*
5	Boveri's picro-acetic (formula 37)	Disrupted	Medium coarse, rippled	1 *B*	Stained	*St*	Intermediate	*In*
6	Champy (formula 84)	Undifferentiated	Vague	0 *D*	Stained	*St*	Even	*Ev*

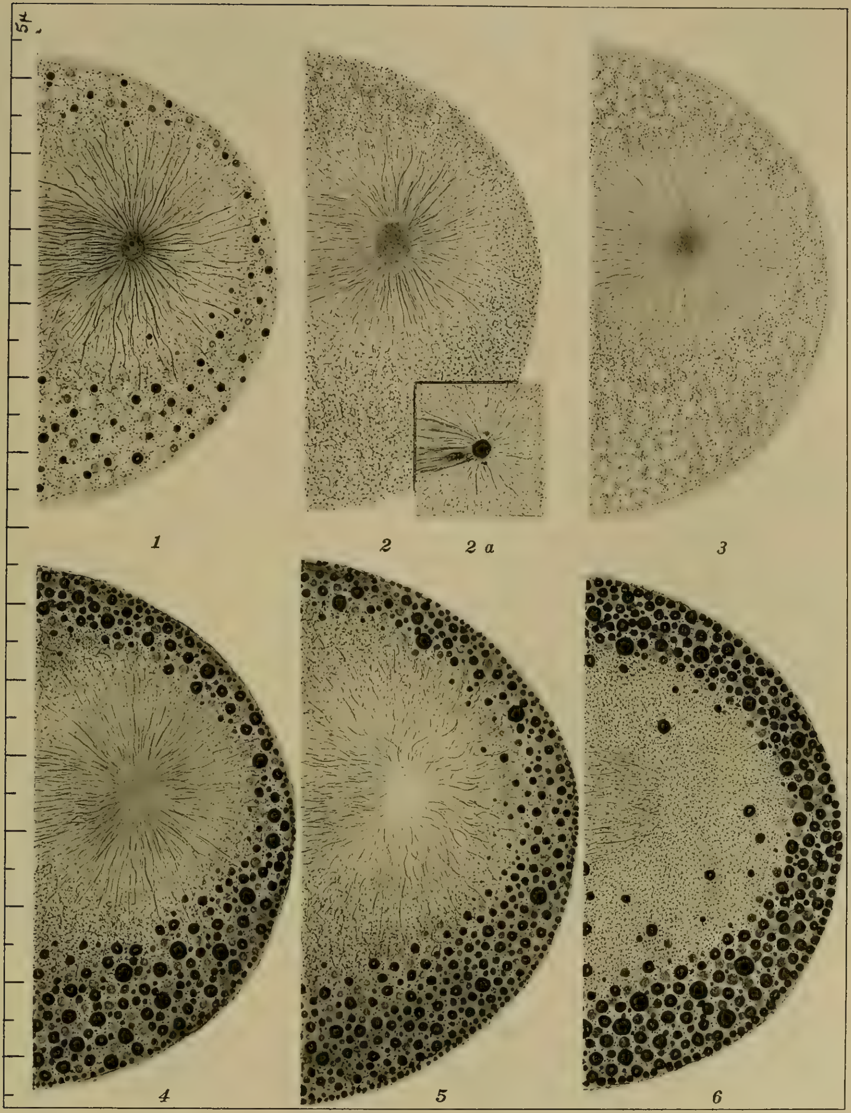
5μ
1
2
2 a
3
4
5
6

TABLE I

The various structural types of asters and cytoplasm demonstrated by different fixatives, used both full-strength and diluted, in Chætopterus eggs, at metaphase, first cleavage. Determination of the percentage by weight of the chemical components, as well as the abbreviation used for each, are discussed page 209; the symbols employed for both astral and cytoplasmic types, including the use of asterisks, are explained in the descriptive material opposite Plate I, and those concerning the cytoplasm are further discussed page 219. *Result:* centrioles are demonstrated only when the fixation also demonstrates very coarse and undulating (almost straight) rays, a condition which is always associated with a disrupted cytoplasm; this is accomplished only by a few reagents, made with certain organic components, diluted to about 10 per cent of their usual concentration. The data of this table are analyzed in various ways in succeeding ones.

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Per-ipheral Bodies			General Cyto-plasm		
							5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group I. Acetic acid, Formaldehyde, Alcohol																		
		Act	*For*	*Alc*	*Others*	*Total*													
1	Acetic acid 1.0 % sol..........	1.0				1.0				√					√				√
2	" 0.75% sol..........	0.75				0.75				√					√*			√	
3	" 0.5 % sol..........	0.5				0.5		√		√				√			√		
4	" 0.25% sol..........	0.25				0.25		√						√			√		
5	" 0.1 % sol..........	0.1				0.1	√							√			√		
6	" 0.01% sol..........	0.01				0.01					√				√			√	
7	Alcohol 20% sol..........			16.0		16.0							√			√			√
8	Alcohol, 50% sol. 99 pts. Acetic acid, 0.5% sol. 1 pt...	0.55		43.0		43.55							√		√				√
9	" 60% strength......	0.33		25.8		26.13				√	√				√			√	
10	" 20% strength......	0.11		8.6		8.71			√					√			√		
11	Formaldehyde 5% sol..........		1.7			1.7						√				√		√	
12	Kahle (modified): 95% Alc., 15 pts; For., 6 pts; Act., 1 pt; water, 31 pts...............	2.0	4.1	22.5		28.6			√						√			√	
13	" 20% strength......	0.4	0.8	4.5		5.7			√						√		√		
14	" 10% strength......	0.2	0.4	2.2		2.8		√	√					√			√		
15	" 5% strength......	0.1	0.2	1.1		1.4	√							√			√		
	Group II. Acetic acid, Formaldehyde, Picric acid																		
		Act	*For*	*Pic*	*Others*	*Total*													
16	Picric acid, sat. sol. 1 pt. Water 2 pts.......			0.4		0.4						√				√			√
17	Bouin (Lee, p. 63)............	5.0	8.2	0.87		14.0					√					√			√
18	" 50% strength......	2.5	4.1	0.43		7.0					√					√			√
19	" 30% strength......	1.5	2.46	0.26		4.2					√					√			√
20	" 20% strength......	1.0	1.64	0.17		2.8				√						√		√	
21	" 10% strength......	0.5	0.82	0.08		1.4	√			√					√		√		
22	" 5% strength......	0.25	0.41	0.04		0.7	√	√							√		√		
23	" 1% strength......	0.05	0.08	trace		0.14		√							√		√		
24	" 0.1% strength......	trace	trace	trace		0.01						√			√			√	
25	Bouin, made with sea water....	5.0	8.2	0.87		14.0					√				√*				√
26	" 20% strength......	1.0	1.64	0.17		2.8	√	√		√					√*			√	
27	" 10% strength......	0.5	0.82	0.08		1.4	√							√			√		
28	" 5% strength......	0.25	0.41	0.04		0.7	√							√			√		
29	" 1% strength......	0.05	0.08	trace		0.14	√							√			√		

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
							5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group II. Acetic acid, Formaldehyde, Picric acid (Continued)																		
		Act	*For*	*Pic*	*Others*	*Total*													
30	Bouin, modified, plus Urea (Carothers' formula)	10.5	5.13	0.91	*Ure* 1.0	17.0					√					√			√
31	Picro-Formol (Bouin without Acetic acid)		8.59	0.92		9.5						√				√		√	
32	" 10% strength		0.86	0.09		0.95						√			√			√	
33	Picro-Sulphuric (Lee, p. 56) (Mayer's formula)			0.4±	*Sul* 3.3	3.7						√				√		√	
34	" 25% strength (Kleinenberg's formula)			0.1±	0.8	0.9						√				√		√	
35	Regaud (Lee, p. 336), 100 pts. Acetic acid 5 pts.	1.04	6.7		*Pot* 2.3	10.0				√						√			√
36	" 10% strength	0.1	0.67		0.23	1.0			√						√*				√
	Group III. Acetic acid, Picric acid																		
		Act	*Pic*		*Others*	*Total*													
37	Picro-Acetic (Lee, p. 56) (Boveri's formula)	1.0	0.4			1.4				√	√					√		√	
38	" 50% strength	0.5	0.2			0.7					√					√		√	
39	" 30% strength	0.3	0.12			0.42	√	√							√*		√		
40	" 20% strength	0.2	0.08			0.28	√	√							√*		√		
41	" 10% strength	0.1	0.04			0.14	√								√*		√		
42	" 5% strength	0.05	0.02			0.07	√	√		√					√*		√		
43	" 1% strength	0.01	trace			0.01							√			√			√
44	Picro-Acetic, made with sea water	1.0	0.4			1.4				√	√					√			√
45	" 50% strength	0.5	0.2			0.7	√			√						√		√	
46	" 30% strength	0.3	0.12			0.4	√								√*			√	
47	" 20% strength	0.2	0.08			0.28	√							√			√		
48	" 10% strength	0.1	0.04			0.14	√							√			√		
49	" 5% strength	0.05	0.02			0.07		√*						√			√		
50	" 1% strength	0.01	trace			0.01							√			√			√
51	Picro-Acetic (form. 37) 80 pts. Alcohol 20 pts.	0.8	0.32		*Alc* 16.0	17.12				√					√				√
52	" 50% strength	0.4	0.16		8.0	8.56				√					√				√
53	" 10% strength	0.08	0.03		1.6	1.71			√	√					√			√	
54	Picro-Acetic (form. 37) with Chloroform to saturation	1.0	0.4		*Clf* 0.6	2.0			√						√*				√
55	" 50% strength	0.5	0.2		0.3	1.0					√					√			√
56	" 10% strength	0.1	0.04		0.06	0.2		√	√							√			√
57	Picro-Acetic (form. 37) 99 pts. Ether 1 pt.	1.0	0.4		*Eth* 0.7	2.1					√				√				√
58	" 50% strength	0.5	0.2		0.35	1.0				√					√				√
59	" 10% strength	0.1	0.04		0.07	0.2			√						√			√	
60	Picro-Acetic (form. 37) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8	0.32		*Plt* 0.2	1.32				√	√					√			√
61	" 50% strength	0.4	0.16		0.1	0.66				√	√					√			√
62	" 10% strength	0.08	0.03		0.02	0.13			√						√				√

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
							5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group IV. Acetic acid, Chloroform, Ether																		
		Act	*Clf*	*Eth*	*Others*	*Total*													
63	Ether 1% sol.			0.7		0.7							√			√		√	
64	Ether, 1% sol. 99 pts. Acetic acid 1 pt.	1.0		0.7		1.7		√*							√				√
65	“ 50% strength	0.5		0.35		0.85		√							√		√		
66	“ 10% strength	0.1		0.07		0.17		√						√			√		
67	Ether-Acetic (form. 64) 80 pts. Alcohol 20 pts.	0.8		0.56	*Alc* 16.0	17.36			√						√				√
68	Ether-Acetic (form. 64) 95 pts. Formaldehyde 5 pts.	0.97		0.67	*For* 1.7	3.3			√						√*				√
69	Ether-Acetic (form. 64) with Chloroform to saturation	1.0	0.6	0.7		2.3			√						√*				√
70	Ether-Acetic (form. 64) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8		0.56	*Plt* 0.2	1.56			√						√			√	
71	Chloroform, saturated solution		0.62			0.62						√				√		√	
72	Chloroform, sat. sol. 99 pts. Acetic acid 1 pt.	1.0	0.61			1.61		√*	√	√					√				√
73	“ 50% strength	0.5	0.3			0.8		√	√	√					√			√	
74	“ 10% strength	0.1	0.06			0.16		√	√	√					√		√		
75	Chlorof.-Act. (form. 72) 95 pts. Alcohol 5 pts.	0.95	0.58		*Alc* 3.94	5.47		√	√						√				√
76	Chlorof.-Act. (form. 72) 95 pts. Formaldehyde 5 pts.	0.95	0.58		*For* 1.7	3.23		√*	√						√				√
77	Chlorof.-Act. (form. 72) with Corros. sublim. to saturation	1.0	0.61		*Sub* 6.5	8.1							√			√			√
78	Chlorof.-Act. (form. 72) 80 pts. Chromic acid, 1% sol. 20 pts.	0.8	0.49		*Chr* 0.2	1.49					√					√		√	
79	Chlorof.-Act. (form. 72) 90 pts. Osmic acid, 2% sol. 10 pts.	0.9	0.55		*Osm* 0.2	1.65						√				√		√	
80	Chlorof.-Act. (form. 72) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8	0.49		*Plt* 0.2	1.49				√						√		√	

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
							5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group V. Acetic acid, Chromic acid, Osmic acid, Potassium Dichromate, Platinic Chloride																		
		Act	*Chr*	*Osm*	*Pot or Plt*	*Total*													
81	Burchardt (Lee, p. 42)	5.4	0.62		*Pot* 1.5	7.5					√					√		√	
82	" 10% strength	0.5	0.06		0.15	0.7				√						√		√	
83	Potassium dichromate 1% sol.				1.0	1.0							√			√		√	
84	Champy (modified) (Lee, p. 353)		0.39	0.39	1.2	1.98						√	√			√			√
85	" 10% strength		0.04	0.04	0.12	0.19						√				√			√
86	Flemming (strong) (Lee, p. 37)	5.2	0.75	0.4		6.35					√					√			√
87	" 50% strength	2.6	0.37	0.2		3.17					√					√			√
88	" 5% strength	0.26	0.03	0.02		0.3				√						√		√	
89	Meves (modif.): 2% Osm. sol., 4 cc.; 1% Chr. sol., 15 cc.; Act., 3 drops	0.79	0.39	0.37		1.55						√	√			√			√
90	" 50% strength	0.39	0.19	0.18		0.76				√	√					√			√
91	" 10% strength	0.07	0.04	0.03		0.15						√				√			√
92	Flemming, without Acetic acid		0.78	0.4		1.18						√				√		√	
93	" 10% strength		0.07	0.04		0.11						√				√		√	
94	Osmic acid 0.2% sol.			0.2		0.2						√				√			√
95	Chromic acid 0.2% sol.		0.2			0.2					√					√		√	
96	Platinic chloride 0.2% sol.				*Plt* 0.2	0.2				√					√			√	
97	Hermann (Lee, p. 39)	5.2		0.4	0.75	6.35				√						√		√	
98	" 10% strength	0.52		0.04	0.07	0.63				√						√		√	
99	Hermann, without Acetic acid			0.4	0.78	1.18							√			√		√	
100	" 10% strength			0.04	0.07	0.11							√			√			√
101	Platin. chlor., 1% sol., 99 pts. Acetic acid 1 pt.	0.99			0.98	1.97			√	√	√				√				√
102	" 50% strength	0.49			0.49	0.98		√	√							√		√	
103	" 10% strength	0.09			0.09	0.19			√	√						√		√	
	Group VI. Acetic acid, Corrosive Sublimate, Nitric acid																		
		Act	*Sub*	*Nit*	*Others*	*Total*													
104	Nitric acid 0.7% sol.			0.7		0.7				√					√				√
105	Corrosive sublimate, sat. sol.		6.5			6.5							√			√			√
106	Kostanecki and Seidlecki (Lee, p. 49)		3.1	1.4		4.4						√				√			√
107	" 10% strength		0.3	0.14		0.44					√					√			√
108	Corros. sublim., sat. sol. 99 pts. Acetic acid 1 pt.	0.94	6.04			6.9				√						√			√
109	" 10% strength	0.09	0.6			0.69				√						√		√	

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
							5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group VI. Acetic acid, Corrosive Sublimate, Nitric acid (Continued)																		
		Act	*Sub*	*Nit*	*Others*	*Total*													
110	Gilson (Lee, p. 49)	0.4	1.9	1.5	*Alc* 4.7	8.5					√					√		√	
111	“ 50% strength	0.2	0.95	0.75	2.3	4.2					√					√		√	
112	“ 10% strength	0.04	0.19	0.15	0.47	0.85					√					√		√	
113	Perenyi (Lee, p. 36)			4.1	*Alc* 25.9 *Chr* 0.16	30.1				√						√		√	
114	“ 10% strength			0.4	*Alc* 2.59 *Chr* 0.01	3.0				√					√*			√	
115	Zenker (Lee, p. 50)	4.6	4.4		*Pot* 2.2 *Sod* 0.87	12.0						√				√			√
116	“ 10% strength	0.46	0.4		*Pot* 0.2 *Sod* 0.08	1.2				√						√			√

When a given component is present in most of the reagents of a group, its amounts are listed in a separate column; but when it occurs only sporadically, it is listed in a column entitled "others."

With two exceptions, all dilutions were brought about by adding distilled water to the reagent. In the case of Bouin's and Boveri's fluids, sea water was also employed (formulæ 25–29 and 44–50).

Reagents which are reported as being used in the usual way, i.e., at full strength, were actually used at about 85 per cent strength, since they were diluted with about 15 parts of eggs and their accompanying sea water at the time of fixation; (from 0.5 to 1 cc. of egg suspension was added to from 4 to 4.5 cc. of fixative in a vial). This situation occurs generally in the fixation of eggs, and, in the case of central bodies in *Chætopterus* eggs, the effect of so slight a dilution may be ignored; only extensive dilution—the addition of more than 25 or 50 parts of water—produces significant changes.

The number of dilutions at which any given combination of components was employed varies considerably. The method of reporting dilutions is as follows: for example, "Bouin, 10 per cent strength" means that 10 cc. of Bouin's fluid was diluted with 90 cc. of water.

In the case of each variously-fixed egg-set only the minimum number of eggs was studied which was necessary to determine the major astral class or classes present. In cases where two or more types occur, no effort was made to determine accurately the relative percentages of each class, since this would have added nothing of significance for the present investigation, and would have necessitated the study of large numbers of cells. Hence, if the examination of 12 metaphase figures in any one egg-set showed that they belong to the same class, with at most one or two exceptions, no further eggs were studied. This is usually the case, and a check in the proper column of Table I indicates the astral type present; any exceptional cases were ignored. If two or more types were found, each in considerable numbers, about 20 eggs were studied, and two or more checks indicate the types occurring, again ignoring the exceptional cases.

Plate I illustrates the major structural classes of central bodies and rays, various combinations of which constitute the astral types; it also shows the different cytoplasmic configurations. The terminology and symbols employed for central bodies and rays are essentially the same as the ones used previously (Fry, 1932, Chart 7, p. 177)[5]; those referring to the cytoplasmic types are described later (p. 219).

III. RESULTS

The Relation Between Central Body Structure and Ray Structure

The data of this investigation, reported in Table I, are in harmony with the results of the previous study. The central body area in *Chætopterus* eggs is very unstable, and exhibits different morphological types after the use of different reagents; each type is always associated with a particular shape and coarseness of rays; and centrioles are demonstrated only if rays are very coarse and undulating (almost straight). This relation is therefore not a phenomenon associated with the use of particular selected reagents, but holds true in the case of the many diverse ones employed in this study.[6] The details of this relationship were discussed in the earlier paper (Fry, 1932, pp. 176–179).

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[5] One change in symbols has been made. Central bodies previously described as "undemarked, even," *i.e.*, stained like the ray area, may have rays which are medium coarse (type 2 *B*), delicate (type 2 *C*) or vague (type 2 *D*) (Fry, 1932, Chart 7). In the latter case, where the radial organization of the aster is doubtful, the central body area is entirely undifferentiated from the peripheral portion; but in the first two the large non-radial centrosome is clearly differentiated from the surrounding ray area, hence the type of center accompanied by vague rays which was previously called "undemarked, even" and was designated by the symbol 2 *D*, is now called "undifferentiated" and is designated by a new symbol, 0 (zero) *D*, which places it at the extreme end of the series (Plate I).

In order to save space in the tables, closely related astral types are grouped together. For example, since the class with an "undemarked centrosome, dense" may be associated with medium coarse rays (type 3 *B*) or with delicate ones (type 3 *C*), the two are grouped together as type 3 *BC*.

In order to determine whether or not minor differences in the exact degree of the coarseness of the rays, which are designated in Table I as "very coarse" (type *A*), are related to differences in the centrioles, six representative egg-sets were chosen for study from the 16 in which centrioles are present. The results are presented in Table II. It is apparent that the rays must not only be very coarse if centrioles are to be demonstrated, but that the heavier they are the larger is the centriole.

Of the 16 formulæ which demonstrate centrioles, some of them do so in practically all of the metaphase figures, while others produce this result in only about half of them, the remainder showing centrosomes without centrioles (4 *AB*). In the sets where all figures have centrioles, most of the cells show typical very coarse rays, or rays even heavier than the typical ones; but in the sets where centrioles are shown only in about half of the figures the rays are usually less coarse, in some cases approaching the medium coarse condition of type *B*. In other words, it would seem that after using reagents which are capable of demonstrating centrioles at all, the percentage of metaphase figures containing centrioles increases as the rays increase in coarseness.

When centrioles are present they do not exhibit the similarity in structural detail from one metaphase figure to another which is associated with typical centrioles. In any one egg-set they vary somewhat in size; they are usually round, but occasionally they are irregular in shape; their contour is smooth in most cases, but rough in others; and the configuration in the large aster may differ from that in the small one of the same figure. Points of this sort cannot properly be considered in a study confined to metaphase, but they will be discussed in the next paper of this group, which will describe their behavior through all phases of various astral cycles. For the same reason, discussion of the phenomenon of the doubling of the centriole when the aster elongates, about the time of metaphase, will also be postponed to that paper.

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[6] Possible exceptions to the usual astral types are produced by three reagents: acetic acid, 0.01 per cent solution (formula 6); chromic acid, 0.2 per cent solution (formula 95); and Gilson's fluid at 10 per cent strength (formula 112). They all demonstrate asters with disrupted centers, many of them with typical rippled rays, "medium coarse," or delicate, such as are associated with these centers (1 *BC*); but in some cases the rays are so coarse and straight as to approach the ray configuration associated with the presence of centrioles. Judging from the character of the fixation and the great variability from cell to cell, these three reagents barely permit coagulation, and the results are probably without significance.

Slightly demarked centrosomes without centrioles (4 *AB*, Plate I, Fig. 2) are demonstrated by 20 of the reagents. They differ in size, shape, physical structure, capacity to take the stain, and the extent to which they are demarked from the ray area, as illustrated in Figs. 1 and 2. Four of the fixatives (formulæ 49, 64, 72, and 76) demonstrate unusually small centrosomes, about 2.5 × 3 μ in size, in contrast to the usual measurements of about 5 × 6 μ. These centers are very variable in their reaction to the dye: many of them stain deeply and resist prolonged destaining, while others on the same slide fail to hold the dye. Most of them are rough and irregular in appearance, but about 10 per cent resemble to some extent the large centriole typical of *Ascaris* eggs (Plate I, Fig. 2*a*). Such centers are listed in Table I in the same column used for the typical large slightly demarked centrosome (4 *AB*) but are designated by an asterisk. In all probability

TABLE II

The relation between coarseness of rays and diameter of centrioles in Chætopterus eggs at metaphase, first cleavage. The table shows six egg-sets in which centrioles are present, listed with respect to differences in the coarseness of their rays. "A" is the typical "very coarse" condition; "A+" indicates rays which are somewhat coarser and "A−" rays which are less coarse. Each centriole diameter reported is an average of 15 measurements in different cells.[7] *Result:* the coarser the rays are, the larger is the centriole.

Formulæ				Ray Coarseness	Centriole Diameter
No.	Components	Name	Dilution		
					μ
48	Acetic-Picric	Boveri	10% strength (sea water)	A+	0.6
41	Acetic-Picric	Boveri	10% strength (distil. water)	A	0.5
15	Acetic-Alcohol-Formol	Kahle	5% strength (distil. water)	A	0.5
5	Acetic		0.1% solution	A	0.3
27	Acetic-Formol-Picric	Bouin	10% strength (sea water)	A	0.4
21	Acetic-Formol-Picric	Bouin	10% strength (distil. water)	A− or B	0.3

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[7] Measurements were made with an ocular micrometer, under such optical conditions that the distance between its lines was 1.6 μ. By the use of a mechanical stage each centriole was placed between the two lines and its diameter estimated by the eye. Obviously such measurements are only approximately correct.

these *Ascaris*-like central bodies in *Chætopterus* eggs, which happen to present an unusually orderly appearance, are but one class out of many, although of no more or less significance than are the larger centrosomes, which are not regarded by cytologists as typical central bodies.

As in the previous study (Fry, 1932, p. 157), some of the "undemarked centrosomes—even" (type 2 *BC*) may contain granules similar to the smaller ones found in the cytoplasm, and certain configurations of them may simulate centrioles. But their rarity, their variation in size, number, and location, make it obvious that they are merely random granules, and they can be dismissed from further consideration.

The Relation Between Astral Type and Cytoplasmic Structure

There are two distinct areas in the cytoplasm of *Chætopterus* eggs—the zone of peripheral bodies, and the general cytoplasm (Plate I). The peripheral bodies occupy a zone about 15 μ wide around the edge of the egg; they are most abundant near the vegetal pole, and least abundant near the animal pole; they are relatively large, the average diameter being about 1.5 μ, although this measurement varies widely. When different fixatives are used this zone exhibits one of three major structural types: first, the peripheral bodies are absent (table symbol: "Ab"); second, they are numerous but remain unstained (table symbol: "Un") after the use of Heidenhain's hæmatoxylin and therefore resemble small vacuoles; (in a few cases where a limited number of the bodies take the stain they are indicated by an asterisk); and, third, the bodies are numerous and deeply stained (table symbol: "St") with Heidenhain's hæmatoxylin.

The general cytoplasm which lies between the peripheral zone and the asters also exhibits three major structural modifications after fixation: first, it has a rough (table symbol: "Ro") and disrupted appearance, as though it had cytolyzed prior to coagulation; second, its appearance is intermediate (table symbol: "In") between that of the preceding and following conditions; and, third, it is even (table symbol: "Ev") and homogeneous in appearance and is largely composed of minute granules which are near the limits of visibility.

Table III presents a resumé of this data from Table I, so arranged that two relationships may be analyzed: First, is there any relation between the appearance of the zone of peripheral bodies and that of the general cytoplasm demonstrated by a given reagent? Second, is there any relation between these various cytoplasmic configurations and the astral type produced at the same time?

As to the first of these relationships, in the six cases when the peripheral bodies are absent the cytoplasm is always "rough"; and of 82

TABLE III

The relation between the astral type and the accompanying cytoplasmic structure demonstrated in Chætopterus eggs at metaphase, first cleavage. The horizontal columns represent the possible combinations of types of the peripheral zone and the general cytoplasm demonstrated by the various reagents; the vertical columns represent the astral types. The numerals indicate the number of formulæ of Table I which demonstrate a given combination.[8] *Result:* centrioles, together with their accompanying very coarse and undulating rays, are demonstrated only when the general cytoplasm is "rough" and looks as though it has cytolyzed, and the bodies of the peripheral zone are absent or unstained.

Cytoplasmic Types		Astral Types						
General Cytoplasm	Peripheral Bodies	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Rough	Absent	2	3	1				
	Unstained	2	2					
	Stained							
Intermediate	Absent							
	Unstained		1	3	3	1	1	
	Stained		1	1	8	6	8	3
Even	Absent							
	Unstained		4	8	5	1		1
	Stained		1		5	7	6	4

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[8] Such tabulations as this are complicated by the fact that the different combinations of chemical components used are represented in some cases by but a single formula, when no dilution was employed, and by two or three or more in cases where dilutions were used. In order to equate these combinations as nearly as possible for the purposes of tabulation the following procedure was used: (1) the combinations which were used in one, two, or three formulæ in which the extent of the dilution differs widely are all reported. Where larger numbers of dilutions were used, only three are reported for each combination of components, as follows: acetic acid, formulæ 1, 3, and 5; Kahle's acetic-alcohol-formol mixture, formulæ 12, 14, and 15; Bouin's acetic-formol-picric fluid, formulæ 17, 18, and 21; and Boveri's picro-acetic reagent, formulæ 37, 38, and 41. (2) Bouin's and Boveri's reagents made up in sea water were not counted in making the tabulations. (3) The Meves formulæ (89–91) were omitted, because they contain the same components as Flemming's (86–88). (4) Where reagents demonstrate more than one astral type, only the one having the most condensed center and the coarsest rays, *i.e.*, the one most closely approaching the structure of type 5 *A*, is reported, and each formula is thus counted but once.

cases in which the peripheral bodies are present, either stained or unstained, the cytoplasm is "intermediate" or "even" in 78, or 95 per cent of them.[9] Thus it is apparent that the fixed structural types of the two cytoplasmic areas are related to each other.

The relation between the various cytoplasmic configurations and the associated astral type is equally clear: if centrioles with slightly demarked centrosomes (5 *A*) are present, the general cytoplasm is always "rough," and the peripheral bodies are either absent or unstained, never stained. But if the centers are uncondensed and not at all like typical central bodies (types 2 *BC*, 1 *BCDE*, and 0 *DE*), the cytoplasm is either "intermediate" or "even"—never "rough"; the peripheral bodies are always present, usually being stained.

It seems probable that the fixed cytoplasmic type which is most like the living condition is that in which the general cytoplasm is "even" and many bodies are present in the peripheral zone. This conclusion is based upon the fact that when the living egg is studied with a high-power water-immersion objective many peripheral bodies are seen, and the general cytoplasm presents a relatively homogeneous appearance. It will be noted that such reagents as those of Champy, Flemming without acetic, Meves, and similar ones, demonstrate "even" cytoplasm and numerous peripheral bodies, and that these fixatives are among those generally regarded by students of cytoplasmic structure as yielding results most like the living condition.

It is concluded, therefore, that the demonstration of centrioles in metaphase first-cleavage figures of *Chætopterus* eggs is associated with the simultaneous disruption of the cytoplasmic structure. In general, the poorer the cytoplasmic fixation is, and the more it deviates from its appearance in the living condition, the more condensed the center is, and the coarser and straighter the rays—and the greater the chances of the demonstration of a centriole.

The Relation Between Astral Type and Egg Volume

Table IV presents data concerning the demonstration of various types of central bodies in relation to the volumes of the eggs and asters. Whenever the astral center is condensed, no matter what its detailed structure is (types 5 *A*, 4 *AB* and 3 *BC*), the average dimensions of the egg are 63.5 × 70 μ, and the volume is approximately 147,000 μ^3. But if the center is not condensed (types 2 *BC*, 1 *BCDE* and 0 *DE*), the average dimensions are 70 × 78 μ and the volume is about 200,000 μ^3. Since the average diameter of the living egg is 97 μ, and its volume about 477,000 μ^3, it is apparent that the technique brings about extensive shrinkage in all cases. However, in the eggs which are least shrunk, whose volume is about 40 per cent of the living condition, the centers are uncondensed, whereas in those shrunk to about 30 per cent of the volume of the living egg the centers are condensed, and some of them have centrioles.

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[9] The type of cytoplasmic structure described as "intermediate" resembles the "even" condition far more than it does the "rough" one. It is also to be noted that the character of the cytoplasmic fixation may vary not only from one egg-set to another supposedly fixed in the same manner, but also from one egg to another within the same egg-set.

Table IV

The relation between astral type and egg volume, in Chætopterus eggs at metaphase, first cleavage. For each astral type there is reported the number of differently fixed egg-sets studied, the average dimensions and volumes of the eggs (based in each case on the measurement of about 50 eggs chosen at random, in about equal numbers, from the different egg-sets), and the average diameters and volumes of the associated asters (these latter measurements being based upon about 25 eggs in each case). Astral measurements are only approximately correct, since it is impossible to determine accurately where the zone of rays ceases. *Result:* condensed centers, some of which contain centrioles, are found only among eggs which are shrunken to a maximum degree as a result of the technique.

Astral Type	5 *A*	4 *AB*	3*BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Number of Egg-Sets Studied	4	7	7	7	8	8	5
Dimensions of Egg (μ)	63×70	61×68	66.6×72	69×75	73×80	69×82	68×76.4
Volume of Egg (μ^3)	143,913	132,207	166,865	186,571	222,752	203,935	184,585
Diameter of Aster (μ)	31	28	31	30	33	Asters Vague or Absent	
Volume of Aster (μ^3)	15,565	11,469	15,565	14,107	18,776		

In all classes of asters with distinct rays, the average diameter of the aster is 30.6 μ and the volume about 15,000 μ^3. Variations in the different classes are relatively minor, and are probably not significant.

The Chemical Conditions of Fixation Necessary to Demonstrate Centrioles

Centrioles are demonstrated only if the chemical components required for their fixation are present in much smaller amounts than those of standard reagents, a condition brought about by dilution.—Among the reagents reported in Table I, 26 were used both at 100 per cent and at 10 per cent strength, or thereabouts. When used full-strength, not one of them demonstrates a centriole, and only four (formulæ 64, 72, 75, and 76) show centrosomes without centrioles (4 *AB*). The results of using them at 10 per cent strength are varied: in 12 cases there is no dilution effect;[10] in the remaining 14, however, a more condensed center and coarser rays are demonstrated when the reagent is employed at 10 per cent strength instead of in the ordinary manner; these results are shown in Table V. Centrioles are demonstrated only after using a few diluted reagents.

TABLE V

The effects upon astral structure of diluting reagents, in Chætopterus eggs, at metaphase, first cleavage. Twenty-six reagents reported in Table I were used both at 100 per cent and at about 10 per cent strength. In 12 there is no dilution-effect. In the other 14 cases, however, there is a dilution-effect, here reported. *Result:* whenever there is any dilution-effect, the astral type demonstrated by the diluted reagents always has more condensed centers and coarser rays than that shown by full-strength fixatives. Centrioles are demonstrated only after the extensive dilution of certain fixatives.

Formulæ		Astral Types Demonstrated at 10% Strength and at 100% Strength						
Components	Form. No.	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Act-Alc-For	12 and 15	10%		100%				
Act (1% sol.)	1 and 5	10%			100%			
Act-Pic	37 and 41	10%			100%			
Act-For-Pic	17 and 21	10%				100%		
Act-Clf-Pic	54 and 56		10%	100%				
Act-Alc-Pic	51 and 53			10%	100%			
Act-Pic-Plt	60 and 62			10%	100%			
Act-For-Pot	35 and 36			10%	100%			
Act-Eth-Pic	57 and 59			10%		100%		
Act-Alc	8 and 10			10%				100%
Act-Chr-Pot	81 and 82				10%	100%		
Act-Chr-Osm	86 and 88				10%	100%		
Act-Sub-Pot-Sod	115 and 116				10%		100%	
Sub-Nit	106 and 107					10%	100%	

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[10] Formulæ 64–66 and 72–74 demonstrate type 4 *AB* regardless of the concentration used; 101–103 demonstrate type 3 *BC*; 97–98, 108–109 and 113–114, type 2 *BC*; 110–112, type 1 *BC*; 31–32, 33–34, 84–85 and 92–93, type 1 *DE*; and 99–100, type 0 *DE*.

The amounts of the components necessary for the demonstration of centrioles fall within narrowly prescribed limits.—Four different combinations of components demonstrate centrioles; each must be used at a specific range of dilution. The results, given in Table VI, show that when centrioles are demonstrated by these four combinations of components the total amount of solutes, regardless of what they are, is from 0.07 per cent to 1.4 per cent by weight. Above and below this optimum range other types of centers are produced, even when the same components are used.

Only certain organic components demonstrate centrioles.—Table VII presents all of the formulæ of Table I in which the percentage by weight of the total solutes falls within the range from 0.07 per cent to 1.7 per cent—a slight extension of the previously described optimum range. They are classified in four groups: (I) those containing the organic components necessary for the demonstration of centrioles; (II) those containing the anæsthetics, chloroform and ether, combined in any way with the preceding components; these two groups include the formulæ which contain only organic components; (III) those containing both organic and inorganic components; and (IV) those containing only inorganic substances.

The results are as follows: *Of the various components used in this study only acetic acid, picric acid, formaldehyde, and alcohol, and only certain combinations of these, demonstrate centrioles.* Thirteen formulæ falling within the prescribed range of total amounts of solutes contain these components, but only four of them fix centrioles (Group I, Table VII). The exact amounts of the components in the various combinations are very important: for example, an acetic-formaldehyde-picric mixture (formula 21), in which the total solute weight is 1.4 per cent, demonstrates centrioles and centrosomes (5 *A*); but when the same components have a total weight of 0.14 per cent (formula 23) the centrosomes are without centrioles (4 *AB*). In this case the greater concentration of components is the more favorable for showing centrioles. On the other hand, an acetic-picric reagent (formula 41) with a total solute weight of 0.14 per cent shows centrioles and centrosomes, but if the total weight of these same components is 1.4 per cent (formula 37) the center is an "even" undelimited centrosome (2 *BC*). In this instance the lesser concentration of components is more favorable for demonstrating centrioles. When acetic acid is used separately the situation is similar: a 0.1 per cent solution (formula 5) shows centrioles and centrosomes (5 *A*), but a 1.0 per cent solution (formula 1) demonstrates large "even" centrosomes (2 *BC*).

TABLE VI

The amounts of components which must be present if centrioles are to be demonstrated in Chætopterus eggs at metaphase, first cleavage
The only four chemical combinations which are capable of showing centrioles were used at various dilutions (Table I). They are grouped here in three classes—those in which the amounts of components are too great for the demonstration of centrioles, those in which the amounts are optimum for that result, and those in which they are too small. *Result:* centrioles are demonstrated only when components are present within certain narrow limits; the total percentage by weight of all solutes present in a reagent must be between about 0.07 per cent and 1.4 per cent.

The Four Reagents Demonstrating Centrioles	The Amounts of Components Required to Demonstrate Centrioles																				
	Too Large							Optimum							Too Small						
	Form. No.	Act	Alc	For	Pic	Total	Astral Type	Form. No.	Act	Alc	For	Pic	Total	Astral Type	Form. No.	Act	Alc	For	Pic	Total	Astral Type
		%	%	%	%	%			%	%	%	%	%			%	%	%	%	%	
Acetic Acid	1	1.0				1.0	2 *BC*	5	0.1				0.1	5 *A*	6	.01				.01	1 *BC*
	2	0.75				0.75	2 *BC*														
	3	0.5				0.5	4 *AB*														
	4	0.25				0.25	4 *AB*														
Acetic-Alcohol-Formol (Kahle)	12	2.0	22.5	4.1		28.6	3 *BC*	15	0.1	1.1	0.2		1.4	5 *A*	No Data						
	13	0.4	4.5	0.8		5.7	3 *BC*														
	14	0.2	2.2	0.4		2.8	4 *BC*														
Acetic-Formol-Picric (Bouin)	17	5.0		8.2	0.87	14.0	1 *BC*	21	0.5		0.82	0.08	1.4	5 *A*	23	.05		.08	.008	.14	4 *BC*
	18	2.5		4.1	0.43	7.0	1 *BC*	22	0.25		0.41	0.04	0.7	5 *A*	24	.005		.008	trace	.01	1 *DE*
	19	1.5		2.46	0.26	4.2	1 *BC*														
	20	1.0		1.64	0.17	2.8	2 *BC*														
Acetic-Picric (Boveri)	37	1.0			0.4	1.4	2 *BC*	39	0.3			0.12	0.42	5 *A*	43	.01			.004	.014	0 *DE*
	38	0.5			0.2	0.7	1 *BC*	40	0.2			0.08	0.28	5 *A*							
								41	0.1			0.04	0.14	5 *A*							
								42	0.05			0.02	0.07	5 *A*							

TABLE VII

The effects upon astral structure of using various combinations of organic and inorganic components, in reagents containing from 0.7 per cent to 1.7 per cent of solutes, in Chætopterus eggs at metaphase, first cleavage. In each group of reagents there are given the formulæ which produce the various astral types, the chemical components of each, and the total percentage by weight of solutes. *Results:* centrioles are demonstrated only by four organic components, and only by certain combinations of them. The presence of anæsthetics and of inorganic components suppresses centrioles. In general, organic components produce coarser rays and more condensed centers than inorganic. (The method of tabulation was that reported in footnote 8, p. 220.)

Reagent Group	Astral Type Demonstrated by Each Reagent						
	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
I Those Containing the Organic Components Acetic, Alcohol, Formol, and Picric	5 Act 0.1%	3 Act 0.5%	53 Act-Alc-Pic 1.7%	1 Act 1.0%	38 Act-Pic 0.7%	11 For 1.7%	
	15 Act-Alc-For 1.4%	23 Act-For-Pic 0.14%		37 Act-Pic 1.4%		16 Pic 0.4%	
	21 Act-For-Pic 1.4%					32 For-Pic 0.95%	
	41 Act-Pic 0.14%						
II Those Containing the Anæsthetics, Ether and Chloroform, and Components of Group I		56 Act-Clf-Pic 0.2%	59 Act-Eth-Pic 0.2%	58 Act-Eth-Pic 1.0%	55 Act-Clf-Pic 1.0%	71 Clf 0.62%	63 Eth 0.7%
		64, 65, 66 Act-Eth 1.7–0.17%					
		72, 73, 74 Act-Clf 1.6–0.16%					

TABLE VII (*Continued*)

Reagent Group	Astral Type Demonstrated by Each Reagent						
	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
III Those Containing Both Organic and Inorganic Components		102 Act-Plt 0.98%	62 Act-Pic-Plt 0.13% 70 Act-Eth-Plt 1.56% 36 Act-For-Pot 1.0% 103 Act-Plt 0.19%	60, 61 Act-Pic-Plt 1.32–0.66% 80 Act-Clf-Plt 1.49% 82 Act-Chr-Pot 0.7% 98 Act-Osm-Plt 0.63% 116 Act-Sub-Pot-Sod 1.2% 109 Act-Sub 0.69%	78 Act-Clf-Chr 1.49% 112 Act-Alc-Sub-Nit 0.85%	34 Pic-Sul 0.9% 79 Act-Clf-Osm 1.65% 91 Act-Chr-Osm 0.15%	
IV Those Containing Inorganic Components Only				96 Plt 0.2% 104 Nit 0.7%	95 Chr 0.2% 107 Sub-Nit 0.44%	85 Chr-Pot-Osm 0.19% 92, 93 Chr-Osm 1.18–0.11% 94 Osm 0.2%	83 Pot 1.0% 99, 100 Osm-Plt 1.18–0.11%

TABLE VIII

The effect of the presence of acetic acid on astral structure in Chætopterus eggs at metaphase, first cleavage. Reagents are reported in pairs, which differ only in that one member of each pair contains acetic acid while the other does not; pairs 3 and 10 differ also in the presence and absence of alcohol. For each pair there is reported the formula number, the components present, and the total percentage by weight of the solutes. The presence of acetic acid is indicated by bold-face type. *Result:* reagents containing acetic acid usually demonstrate coarse rays and more or less condensed centers, and those without acetic acid do not; centrioles and their accompanying very coarse rays are demonstrated only when acetic acid is present.

Pair Nos.	Astral Type Demonstrated by Each Reagent						
	5 A	*4 AB*	*3 BC*	*2 BC*	*BC*	*1 DE*	*0 DE*
1	39 **Act-Pic** 0.42%					16 Pic 0.4%	
2	22 **Act-For-Pic** 1.7%					32 For-Pic 0.95%	
3	15 **Act-Alc-For** 1.4%					11 For 1.7%	
4		65 **Act-Eth** 0.85%					63 Eth 0.7%
5		73 **Act-Clf** 0.8%				71 Clf 0.62%	

TABLE VIII (*Continued*)

Pair Nos.	Astral Type Demonstrated by Each Reagent						
	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
6			103 Act-Plt 0.19%	96 Plt 0.2%			
7				108 Act-Sub 6.98%			105 Sub 6.5%
8				98 Act-Osm-Plt 0.63%			100 Osm-Plt 0.11%
9				9 Act-Alc 26.1%			7 Alc 16.0%
10					111 Act-Alc-Sub-Nit 4.25%	106 Sub-Nit 4.4%	
11						89 Act-Chr-Osm 1.55% 92 Chr-Osm 1.18%	

The presence of the anæsthetics, chloroform and ether, prevents the demonstration of centrioles.—An acetic-picric mixture (formula 41) with a total solute weight of 0.14 per cent demonstrates centrioles and centrosomes; but if this same combination, in slightly differing amounts, has either chloroform (formula 56) or ether (formula 59) added to it, the total amounts of solutes in both cases being 0.2 per cent, centrosomes without centrioles are shown (4 *AB* and 3 *BC*). Similarly, acetic acid used separately in a 0.1 per cent solution (formula 5) demonstrates centrioles and centrosomes, but if either chloroform (formula 74) or ether (formula 66) are added, the total solutes being 0.16 per cent and 0.17 per cent respectively, centrioles are not shown, only the centrosome being present (4 *AB*).

The presence of inorganic components prevents the demonstration of centrioles.—In general, the demonstration of dark and condensed centers is more likely to result from using organic components than inorganic ones. For example, if acetic acid is used in a 0.1 per cent solution (formula 5), the astral type produced has a centriole and centrosome, but the addition of platinic chloride, the total solute weight being 0.19 per cent (formula 103), or of chromic and osmic acids, the total solute weight being 0.15 per cent (formula 91), prevents the demonstration of both centriole and demarked centrosome, producing instead types 3 *BC* and 1 *DE* respectively. Of the 14 formulæ of Table VII which demonstrate the most condensed centers (5 *A* and 4 *AB*), 13 of them contain only organic components, the single exception involving the presence of platinic chloride in formula 102.

Without an exception, the demonstration of any kind of a dark and condensed center requires the presence of organic components in the fixative. Of 20 formulæ which demonstrate such centers (5 *A*, 4 *AB*, 3 *BC*), 15 contain only organic components, and the other five contain both organic and inorganic (Table VII).

The formulæ of Table I which have total amounts of solutes in excess of the range just reported were analyzed in the same manner. While none of them demonstrate centrioles, the general situation concerning the effect upon the fixed astral center of using organic components in contrast to inorganic ones is the same.

Acetic acid is the component most favorable for the demonstration of centrioles and condensed centers generally.—The data of Table I were analyzed with reference to a possible specific effect of the presence of each component, regardless of the occurrence of other components. The only result of significance is in the case of acetic acid. Among the formulæ listed in Table I are 11 pairs which are similar both as to the components present and the total amounts of the solutes; the members of each pair differ only in that one contains acetic acid and the other does not (in two cases the difference involves the presence or absence of acetic acid plus alcohol). Table VIII reports the astral types demonstrated by these pairs of reagents.

With one exception the presence of acetic acid causes the demonstration of an astral type with a more condensed center than that produced by a similar reagent without acetic acid; the exceptional reagent (pair 11), which is a chrome-osmic mixture, disrupts the centers whether acetic acid is present or absent. Of the 11 reagents which contain acetic acid, nine demonstrate more or less condensed "filled" centers, three of them with centrioles, and only two have disrupted centers. On the other hand, of the 11 similar reagents which do not contain acetic acid, only one demonstrates a "filled" center, and that is of the least condensed type (2 *BC*); the remaining ten have either disrupted or undifferentiated centers. Furthermore, it is significant to note that acetic acid is the only component which occurs in all four of the combinations which demonstrate centrioles; in one of them it is used alone and in the other three it is combined with other components.

Centrioles are more readily demonstrated if sea water is used to dilute the reagent in place of distilled water. Of the four combinations of components which demonstrate centrioles when diluted with distilled water, two were also diluted with sea water—Bouin's acetic-formol-picric mixture and Boveri's acetic-picric fluid. In both cases the full-strength reagents were made up with sea water, the picric acid being dissolved in it (which may possibly have affected the amount which went into solution). With both reagents the following results hold true: First, when sea water is used centrioles are demonstrated by a less dilute mixture (compare formulæ 17–24 with 25–29, and 37–43 with 44–50). Second, when sea water is used centrioles usually occur in all metaphase figures within the range of dilution which shows them at all; only about half of them show centrioles when distilled water is employed (Table I). Third, sea water dilution demonstrates a somewhat larger centriole than does distilled water (Table II). Finally, in the case of Bouin's fluid the use of sea water widens the range of dilution within which centrioles are demonstrated; this does not hold true of Boveri's reagent, although the position of the range is slightly shifted (Table I).

IV. Discussion

The previous study in this group (Fry, 1932) showed that in *Chætopterus* eggs at metaphase, centrioles are demonstrated only by a few of the many modifications of Boveri's picro-acetic fluid used, and only when the reagent is so diluted that the chemical components are present in certain small amounts. The present study confirms and extends that conclusion: centrioles are demonstrated by only four different chemical combinations, out of 48 employed; certain organic components must be present, and the usual reagents must be diluted with 70 to 95 parts of water.

Although these facts appear to be well established, their interpretation is difficult, if not impossible. If many different combinations of components demonstrated centrioles when used in small enough amounts, the situation would be simplified, for we might then be dealing with the results of poor fixation, brought about by using reagents in amounts so small as to barely permit coagulation; but the fact that only four combinations are effective when diluted is hard to explain. If the four organic components which demonstrate centrioles had any characteristic in common which might be of significance for coagulation—if, for example, they were all reducing agents, or related acids—an explanation of the situation might be suggested, but such is not the case. Perhaps the result is due primarily to the presence of acetic acid, since it can demonstrate centrioles when used alone, and is present in the four combinations which show them; but why then should it be effective only when used in certain small amounts, and not when the slightest increase is employed (formulæ 1 to 6)? [11]

The fact that there is a relationship between the demonstration of a given type of central body and the simultaneous demonstration of certain specific configurations of other cell structures is not affected, however, by the inability to explain the coagulation phenomena in physico-chemical terms, and it is quite possible that an understanding of this relation may add to our knowledge of central bodies.

The previous investigation showed that the demonstration of centrioles in *Chætopterus* eggs requires the presence of very coarse, undulating rays, but that conclusion was based upon the use of various modifications of a single reagent, picro-acetic. The possibility that other fixatives might have different effects has been eliminated by the present study, in which the relation between the structure of the central body and that of the rays is found to be constant in all respects after the use of 116 formulæ of diverse chemical composition.

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[11] The various components were analyzed in terms of their molar solutions, but the results were not significant. The fact that dilution with sea water is more favorable for the demonstration of centrioles than dilution with distilled water may be due to a difference in the hydrogen ion concentration (cf. Fry, 1932, pp. 171–172). Differences in the osmotic pressure of the reagents probably is not a factor, since complete fixation occurs within the first second, and the plasma membrane is therefore instantly killed (cf. Fry, 1932, pp. 173–176).

Furthermore, this relation is now extended to include the type of cytoplasmic structure shown, as well as the size of the cell. If the coagulation is such that the fixed egg is shrunk to about 40 per cent of the volume of the living one, and the cytoplasmic structure is somewhat like that of the living condition, astral rays are usually either vague or delicate and the center is never condensed; but if the process of fixation shrinks the egg to about 30 per cent of the volume of the living one, and disrupts the cytoplasm, the rays are usually coarse and the center more or less condensed. Only under the latter conditions are centrioles demonstrated, and then but rarely.

The two interpretations discussed in the previous paper again present themselves—with more data now available on both. In the first place, we may be dealing with a centriole so exceedingly difficult to demonstrate, and shown under such unusual conditions of fixation, that its behavior in this respect constitutes a new chapter in the history of this cell component. For some reason, as yet unknown, its demonstration requires the fixation of rays which are at the same time undulating and very coarse—if either condition occurs without the other no centrioles are shown. According to this point of view it would be assumed that the chemical composition of the centriole and the rays differs so widely from that of the cytoplasm that their proper fixation necessarily disrupts the cytoplasm and shrinks the egg to the maximum extent. The unusual difficulty in demonstrating *Chætopterus* centrioles might then explain their variability in detailed structure from cell to cell under the rare conditions when they are shown.

The other interpretation of these phenomena assumes that the central area of the living aster is largely composed of the same kind of materials as are the rays; that diverse fixatives modify the detailed configuration of the entire aster; that the morphology of the fixed center is related to the way in which the peripheral ray area is coagulated. In this event, it is further assumed that when extensive dilution reduces the components of reagents to very small amounts, the fixatives are then approaching the lower limits of concentration which will permit any coagulation at all. When this happens, under certain chemical conditions the cytoplasm is disrupted, the cell is shrunken to a maximum degree, the ray configuration is unusually coarse, and the focal area of such rays is consequently unusually condensed. Under such conditions, the centers may take various forms: some resemble large slightly demarked centrosomes; some are smaller, stain deeply, and look somewhat like *Ascaris* central bodies; and in others the center looks like a typical centriole surrounded by a centrosome. But all of these may be "focal bodies" (Fry, 1932, p. 181), *i.e.*, the results of the coagulation of areas where rays or spindle fibers converge. From this point of view, the variability in central body structure from one cell to another on the same slide is explained by the assumption that the exact morphology of any center depends upon the structure of the associated rays, which, in turn, depends upon the extent of the dilution to which the individual egg was exposed at the time of fixation (Fry, 1932, pp. 164–165). If this interpretation is correct, the class of fixed *Chætopterus* eggs which is most like the living condition has a homogeneous undisrupted cytoplasm, numerous peripheral bodies, and relatively delicate rays accompanied by a large uncondensed central area (2 *BC*, 1 *BC* or 0 *DE*, Plate I, Figs. 4, 5 and 6). In this event, the demonstration of centrioles in *Chætopterus* eggs is probably a result of such poor fixation that the egg is grossly distorted, and we are dealing with coagulation artifacts, which are especially likely to occur at areas of focalized rays or fibers.

Which of these two interpretations is most applicable to the data? The answer to that question depends upon our criteria for determining "good" and "poor" fixation. No single procedure can be followed because different cell components exhibit such varying structural stability after coagulation. For example, blepharoplast-centrioles and chromosomes are so similar from cell to cell at any given stage, even after using diverse fixatives, that to determine the class of coagulation products which most resembles the living condition presents no difficulty in many investigations. On the other hand, the situation is very different if one is studying the detailed structure of the cytoplasm, or the exact configuration of astral rays, or the morphology of the central body in many egg cells. The central bodies of *Chætopterus* eggs exhibit various structural types—so many as to constitute a continuous series extending from a condensed and delimited condition to an uncondensed or disrupted one. In such a case, how shall the "best-fixed" class be determined?

The usual procedure in cells where the central bodies exhibit considerable diversity of structure after fixation is to select as "best-fixed" those which contain minute sharply demarked bodies at the astral centers, resembling the central bodies frequently seen in spermatocytes. *If the demonstration of a centriole-like body at the astral center is proof per se that it actually is a centriole, such procedure is valid.* In that case it is unnecessary to report the relative number of cells which contain centrioles, and the other classes can legitimately be dismissed as "poorly fixed" without any attempt to discover why they differ from the "best" class, and without analyzing possible interrelations between the classes. Furthermore, such evidence may be considered depend-able even when the demonstration of centrioles is the result of an unsuspected and uncontrolled modification of the technique, which makes repetition of the result impossible when the technique is used in the usual way, a situation which has occurred in the investigation of *Chætopterus* centrioles, and is discussed in the previous paper (Fry, 1932, pp. 149–150 and 162–164).

The danger in assuming that the demonstration of a centriole-like body proves the existence of a real centriole lies in the fact that the coagulation of focalized rays and spindle fibers may possibly result in the production of a structure which simulates a centriole but is an artifact. Since the chemical composition and physical structure of the center of an aster or the tip of an anastral spindle differs from the peripheral part, is it not possible that it may coagulate differently? Furthermore, it has now been demonstrated that the structure at the focal area is in some cases extremely variable, and that its morphology is related to that of the converging rays or fibers. This has been shown in several studies: in cytasters, sperm-asters, and first-cleavage figures of *Echinarachnius* eggs (Fry, 1928, 1929); in metaphase first-cleavage figures of *Chætopterus* eggs (Fry, 1932, and the present paper); and in the anastral mitotic figures of brain cells in *Squalus* embryos (Fry and Robertson, 1933). In the latter case, a relationship is also demonstrated between the structure of the mid-bodies and the detailed configuration of the fibers of the mid-region of the spindle which are focalized by the cleaving cell. On the basis of such data the suggestion has been advanced that when areas of focalized rays and fibers are coagulated, structures may be produced which simulate central bodies but which are nothing but phenomena of focalization.

Difficulties of interpretation such as these explain the attitude expressed in various quarters—that the study of fixed cells frequently yields data of little or no value, due to the distortion of cell structure frequently resulting from coagulation, and to our lack of knowledge of the physical chemistry of the process. In a measure this attitude is justified; certainly no one would study fixed cells, for the reasons just given, if the desired information could possibly be secured by studying living cells. But in many cell types the structure in question is discernible only vaguely or is totally invisible, and the only possible method of investigation at present available is the use of fixed, sectioned, and stained material. To discard the method because of its dangers is to forego securing significant facts which can be obtained in no other way. Such a method is properly used, however, only when adequate steps are taken to guard against its dangers, and the precautions required differ in the case of the various cell structures.

The data presented in this and the preceding study of *Chætopterus* central bodies are still too incomplete to permit a decision as to whether they are atypical ones or focal artifacts, because the work has so far been confined to metaphase, first-cleavage figures. The next paper on this subject will describe the behavior of *Chætopterus* centrioles through all of the maturation stages of the egg, and from the early history of the sperm-aster through late cleavage. Some of the asters are large and show marked changes in ray configuration during a single mitotic cycle, elongating during the process; others are small and show but slight structural changes. The behavior of the centriole under these various conditions provides significant additional evidence concerning its nature.

V. Resumé

The structure of central bodies in *Chætopterus* eggs at metaphase, first cleavage, was studied after fixation with 116 reagents, which represent 48 different combinations of chemical components; all were used at the usual concentrations, and most of them at various dilutions.

Centrioles are demonstrated by only four of the combinations, composed of one or more of the organic substances, acetic acid, picric acid, formaldehyde, and alcohol. The only component present in all four is acetic acid. The presence of the anæsthetics, chloroform and ether, as well as of numerous inorganic substances, prevents the demonstration of centrioles. In the case of the four effective combinations the components must be present within very small and narrowly limited amounts, differing in the case of each combination, and requiring a dilution of the usual reagent with 70 to 95 parts of water. Dilution with sea water is more effective for the demonstration of centrioles than the use of distilled. The data of the present study are in harmony with those of the previous one, which was confined to modifications of a picro-acetic mixture.

As in the previous work, various types of central bodies are demonstrated, each associated with a specific astral configuration, which varies as to the coarseness and shape of the rays. Centrioles are shown only when rays are very coarse and undulating (almost straight), which occurs but rarely.

The demonstration of centrioles and very coarse undulating rays is always accompanied by a disrupted cytoplasm which looks as though it had cytolyzed before it coagulated.

After the use of all reagents the eggs are extensively shrunken. Those which are about 40 per cent of the volume of the living egg have uncondensed centers; those shrunk to about 30 per cent have condensed ones and some of these contain centrioles.

When centrioles are present they do not exhibit the orderly behavior of typical ones. In some egg-sets they occur in practically all metaphase figures; in others they are found in about 50 per cent of them. They differ, from one metaphase figure to another on the same slide, in size, shape, contour, and other points. The larger centrosomes also vary in every respect; after the use of a limited number of reagents, about 10 per cent of them look somewhat like the central body typical of *Ascaris* eggs.

The two interpretations discussed in the previous study again present themselves, but with additional data now available. On the one hand, we may be dealing with a very atypical centriole, different from any previously described, which can be demonstrated only under rare and peculiar conditions of dilute fixation, and only when a specific ray configuration is also shown; the cytoplasm is disrupted and the egg is shrunken to a maximum extent when this condition is produced. On the other hand, all of the various classes of central bodies demonstrated may be phenomena of focalization; the manner in which the peripheral part of the rays is coagulated may determine the morphology of their focal area; and when the conditions of fixation produce very coarse rays, the "focal body" is correspondingly condensed, happening sometimes to simulate a centriole.

VI. BIBLIOGRAPHY

Fry, Henry J., 1928. Conditions Determining the Origin and Behavior of Central Bodies in Cytasters of Echinarachnius Eggs. *Biol. Bull.*, **54**: 363.

Fry, Henry J., 1929. The So-Called Central Bodies in Fertilized Echinarachnius Eggs. I. The relationship between central bodies and astral structure as modified by various mitotic phases. *Biol. Bull.*, **56**: 101.

Fry, Henry J., 1932. Studies of the Mitotic Figure. I. Chætopterus: central body structure at metaphase, first cleavage, after picro-acetic fixation. *Biol. Bull.*, **63**: 149.

Fry, Henry J., and C. W. Robertson, 1933. Studies of the Mitotic Figure. II. Squalus: The behavior of central bodies in brain cells of embryos. *Anat. Rec.*, **56**: 159.

Mead, A. D., 1898. The Origin and Behavior of the Centrosomes in the Annelid Egg. *Jour. Morph.*, **14**: 181.

Wilson, Edmund B., 1930. The Question of the Central Bodies. *Science*, **71**: 661.

STUDIES IN THE LIFE HISTORIES OF EUGLENIDA

III. The Morphology of Peranema trichophorum Ehrenberg, with Special Reference to its Kinetic Elements and the Classification of the Heteronemidæ

JAMES B. LACKEY

Department of Water and Sewage Research, N. J. Agricultural
Experiment Station

Peranema trichophorum (Ehrenberg) emend Stein is perhaps the most common of the larger, free-living, holozoic flagellates. It is easily cultivated in the laboratory, thriving on several media, and thus lends itself readily to experimental purposes.

The genus *Peranema* was established in 1841 by Dujardin, to receive organisms of the genus *Pyronema,* a term employed by him in 1836, but found to be already in use. He described three species, viz.: *P. globulosa, P. protracta,* and *P. virescens,* giving a figure of the first. Ehrenberg's description in 1838 is accompanied by nine figures which conform to the description of *P. protracta* by Dujardin, whereas the figure given by Dujardin does not resemble a *Peranema.*

Stein in 1878 placed the species *trichophorus* of Ehrenberg in the genus founded by Dujardin. He made an extensive investigation of the organism, describing a single flagellum and a single rod-organ. He also pictured the division of the animal. After him, a number of investigators, including Klebs (1883–1892), Fisch (1885), Butschli (1887), Senn (1900), Hartmann and Chagas (1910), Rhodes (1926), Hall and Powell (1928), and Brown (1930) recorded observations on the organism. The work of Klebs was especially careful. He described very accurately the mouth of the animal, two rod-organs, and a single flagellum. As a result of his studies, he divided the Euglenoidina of Butschli into three families: Euglenida, Astasiida, and Peranemida, which were respectively holophytic, saprophytic, and holozoic in nutrition. The Peranemida he divided into the subfamilies Peranemeæ, having a single flagellum, and Heteronemeæ with two flagella.

Senn changed this slightly in 1900, principally in establishing a number of subfamilies in his family Peranemaceæ. Dangeard in 1900 again instituted minor changes in terminology. Lemmermann, in 1913, followed Senn in naming the families, and Walton, in 1915, followed the same general scheme, although he traced the family names Euglenidæ, Astasiidæ, and Peranemidæ to Stein, Butschli, and Ehrenberg respectively. Doflein (1916) retained the three families Euglenidæ, Astasiidæ, and Peranemidæ.

It will be noted that all classifications into families since Klebs have been largely dependent on modes of nutrition, symmetry perhaps playing a secondary part. Calkins in 1926 dropped the Peranemidæ. Those organisms possessing chloroplasts he placed in the family Euglenidæ, including here also a few questionable colorless forms. In the Astasiidæ, he included the colorless forms having a single flagellum. For colorless forms having two flagella, he created a new family, the Heteronemidæ.

Descriptive work on *Peranema trichophorum* shows an animal with a single flagellum and one or two rod-like structures in the mouth region. Dangeard (1901) shows two of these rods. Walton describes one; Lemmerman figures two, but speaks of a single parabasal body. Hartmann and Chagas show two, Hall and Powell two; Brown two; Doflein has a brief description of the family and the species, accompanied by an original figure. He shows a single flagellum, bifurcated at its base, the two roots ending in basal bodies, located against one of the two rod-organs ("stabapparat"). Calkins' description of the animal is largely in his key. He figures a single rod-organ, calling it a parabasal. Hall (1926) and Hall and Powell (1928) also describe the flagellum as single, and non-bifurcated at its base, while Brown (1930) shows it to be bifurcated at its base. Other workers describe the flagellum as single, vibratile at its tip, emerging from the mouth region and with a basal body. The animal is said to be spindle-shaped, tapering anteriorly, 20 to 70 microns long and 10 to 25 wide. It has a pronounced pharynx and reservoir into which a contractile vacuole opens. The thin pellicle is faintly striated spirally. Movement is creeping, although the animal swims at times. It frequently changes the shape of its body, becoming rounded up or forming an irregular ball. Nutrition is holozoic according to Walton and Lemmermann; saprophytic according to Doflein; and is not mentioned by Calkins.

While working on the life cycle of the animal (Lackey, 1929), it was noted that it appeared to have two flagella, so more careful cytological studies have been made.

METHODS

The organisms were killed and fixed on coverslips which were either floated on the cultures or immersed until the animals crept on them. Schaudinn's fluid, Flemming's strong solution with and without acetic acid, and Bouin's fluid were used as fixatives. Of several stains employed, iron hæmatoxylin and Regaud's hæmatoxylin with eosin as an occasional counterstain were the most successful ones. Living animals were studied under oil in hanging drop preparations.

FINDINGS AND DISCUSSION

A number of changes in the present description of the animal and some new characteristics which are regarded as diagnostic are enumerated. The length of the animal often exceeds 70 microns, specimens as long as 90 microns having been encountered. Individuals under 50 microns were not found, although many "wild" specimens were examined, in addition to those from the cultures. A very small form, 25 to 35 microns long and 10 to 12 wide, was often found in both stock cultures and material collected from various localities. While it appears to be identical with the larger form on visual examination, no mass cultures could be procured from isolations of it.

Bilateral symmetry is very evident; the mouth is on the ventral surface, and of peculiar shape, perhaps most comparable to a tuning fork whose prongs are short and pointed, the base of the fork being at the anterior end of the animal (Fig. 1). Klebs (1892) shows this very clearly; according to Hall (1926) the cytostome is subterminal.

EXPLANATION OF PLATE I

FIG. 1. A diagrammatic sketch of the anterior end of *Peranema trichophorum* viewed from the ventral side showing the various organelles and their relation to each other. No connection between any of these and the nucleus has been found during interkinesis.

A. Primary, anteriorly directed or swimming flagellum.
B. Curved cytostomal element.
C. Mouth opening.
D. Internal gullet from mouth to reservoir.
E. Rod organs.
F. Fibril from blepharoplasts presumably to curved cytostomal element.
G. Two blepharoplasts.
H. Reservoir.
I. Secondary, posteriorly directed or trailing flagellum.
J. Contractile vacuole.

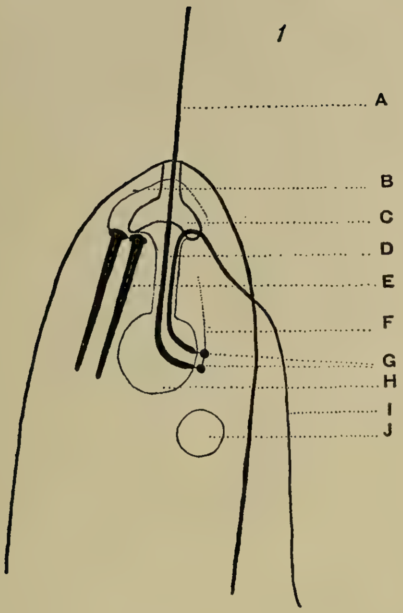
FIG. 2. A similar sketch of the anterior end of the animal as seen from the side. The two rods may either form a V or appear as single in such a view.

FIG. 3. A diagrammatic drawing of a *Peranema* about the metaphase in division. The cytoplasm has been stippled to bring out the nucleus and the gullet-reservoir more clearly. The chromosomes of the dividing nucleus are not shown, but the elongating endosome and the persistent nuclear membrane are shown. The nuclear membrane appears to be pulled out at the poles and is attached to the blepharoplasts, but there is no connection of any kinetic or cytoplasmic organelles to the endosome. Four rod-organs are seen to be arising *de novo* in the cytoplasm, the original pair having disappeared.

FIG. 4. A similar drawing of a telophase stage. The four flagella are clearly evident, having elongated sufficiently to emerge through the mouth. In this and the preceding figure each is seen to originate from one of the blepharoplasts.

PLATE I

1


A
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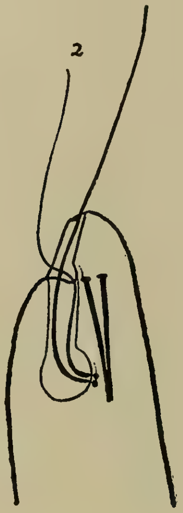
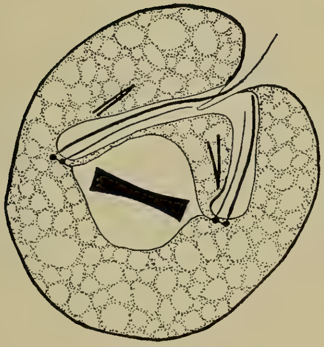
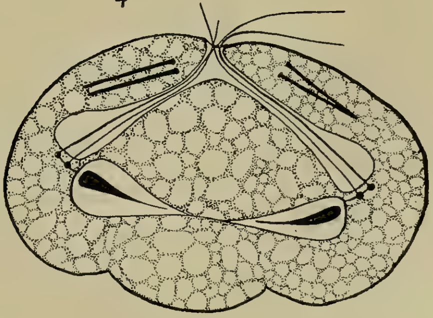
2



3



4



The animal has two flagella. The existence of two has been unrecorded until now, although Lemmermann hints at the existence of a second (see Pascher; Die Süsswasserflora, Heft 2, Flagellatæ 2, page 117), and Conn and Edmondson figure an *Astasia trichophorum* with two flagella which may be *Peranema*. Failure to see the second flagellum is possibly due to a sublimate fixer, which often works havoc with delicate protoplasmic extrusions of this nature, whereas osmic fixatives leave them apparently intact. The larger flagellum is used for swimming or creeping, and only its distal quarter is vibratile. The smaller is a trailing flagellum and is about two-thirds as long as the body of the animal. Almost invariably it is held or trails naturally under the ventral surface of the body which often touches the substratum only at the sides, leaving an arch beneath the animal which would tend to hide the trailing flagellum. The writer has never been able to get more than a faint idea of its existence in living animals.

Both flagella emerge from the side wall of the reservoir (Figs. 1, 2), at right angles to the main axis of the animal. About the middle of the reservoir they turn sharply, paralleling the main axis, and pass up through the gullet into the mouth. The primary one extends normally straight out through the anterior troughlike portion of the mouth, so that in the living animal it appears to originate at the very tip of the animal. The secondary one emerges from one of the two arms of the mouth, turning backward. Both flagella have a very thick sheath as they leave the reservoir wall, but the primary is the larger. Both taper gradually, the sheath of the primary tapering to an end about where this flagellum becomes vibratile, while the sheath of the secondary is apparently lost in the gullet. The sheaths are either striated or uneven. The axial fibril of each flagellum terminates in a round blepharoplast adjacent to the reservoir wall. Hall (1926) (1928), speaking of a single flagellum (the principal one), says a rhizoplast is traceable to a granule which he terms the centrosome at the nuclear membrane. Hall and Powell state that this centrosome and not the blepharoplast serves as the pole in division. They find in the prophases a pair of granules or extranuclear centrosomes on the nuclear membrane connected by rhizoplasts to the blepharoplasts, and consider both blepharoplast and centrosome to be genetically continuous.

No amount of examination by the writer has shown a granule in the interphase on the nuclear membrane. There is often a vesicle above the nucleus, but no granule or rhizoplast in this region. Furthermore the old flagella are thrown off or absorbed in division and new ones arise, just as the writer has shown in *Entosiphon* (1929). This is easily seen in the living animal and the writer has demonstrated it in stained preparations. The new ones grow out from two granules at each pole of the dividing nucleus, and these granules are the blepharoplasts. With the disappearance of the old flagella in the prophase the blepharoplasts each divide, and with nuclear elongation they migrate also, forming the poles of the dividing nucleus, as seen in Figs. 3 and 4. The animal is rounded up, although intensely metabolic, during division, and constriction of the cell does not begin until late; consequently the two gullets and reservoirs are close to the ends of the dividing nucleus. The heavy nuclear membrane remains intact and there is a definite orientation of the blepharoplasts as polar granules. But there is no origin of these granules from the endosome or any other part of the nucleus. These granules should therefore be termed centroblepharoplasts.

This account is in accord with that of Hall and Powell for one set of granules, but not for two sets. Nevertheless, it seems to the writer that to find a division of the blepharoplast and a division of an extranuclear granule which serves as a centriole or centrosome, and to assume a growth of a second rhizoplast (since one rhizoplast is found in the prophases by Hall and Powell) from the new centrosome to the new blepharoplast is a reductio ad absurdum. No division of the rhizoplast seems to be assumed, however. In reality there is no necessity for either assumption. The condition these writers show in Plate I, Fig. 4, is typical of the vegetative condition; nor do they show a separate blepharoplast and centrosome in any figures of their two plates, except possibly in Fig. 3. And as they show in the relative positions of gullet and dividing nucleus, only a very slight migration of the blepharoplasts would be necessary for these organelles to occupy polar positions. The actual occurrence is the division of the blepharoplasts in the prophase; the outgrowth of new flagella from them and their further functioning as extranuclear division centers, as shown by the writer in Figs. 3 and 4. They are wholly independent of the endosome in interkinesis and in division.

Brown (1930) shows very clearly the relationships of the blepharoplasts to the dividing nucleus in his figures. In his account he states that strands pass from these centroblepharoplasts to the endosome. From the number of these strands he shows, it might be concluded that they represent a spindle. In Fig. 6, pl. 19, for example, they are obviously invading the area occupied by the chromosomes as well. He is not clear on the mechanism of formation of a new kinetic complex: "During the prophase the basal granules divide twice to form new basal granules; out from these grow axial filaments. Each of these filaments unites with one of the old axial filaments to produce a new flagellum. A part of the flagellum thus persists. . . ." If he means that *each* basal granule divides once, it is necessary to postulate a splitting apart of the two roots of the old flagellum. However, we have definitely stated that the old flagella are discarded in the early stages of division. If we accept the existence of a second flagellum, all accounts experience less difficulty in explaining their figures. Furthermore, it is hardly possible that Hall and Powell, Brown, and the writer would make such diverse observations with regard to the flagellum blepharoplast complex except on the basis of the existence of a hitherto unreported second flagellum. The evidence here, as elsewhere (see Hall and Powell), shows no nuclear origin for the kinetic elements, but instead a genetic continuity, which is to be expected for an organelle serving as a centriole and presumably homologous with the centrioles of higher forms.

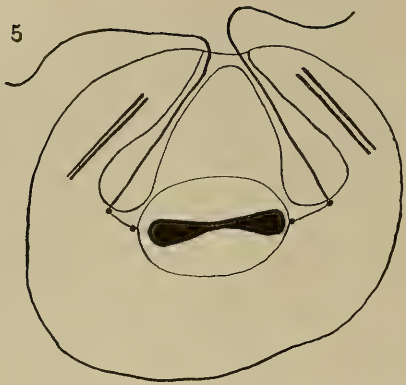
There are also two rod-organs. They are practically parallel to the main axis of the animal, extending from about the region of the mouth to a point slightly below the reservoir. Often their posterior ends are close together, so that they form a narrow V. Like the blepharoplasts they stain well with iron hæmatoxylin. At the upper end each terminates in a slight knob alongside the gullet (Fig. 1). From the knob of one of them a thin fibril extends in a curve over the mouth and down in the general direction of the blepharoplasts. It has been impossible to trace the entire course of this fibril. It is probable that it is connected to one of the two blepharoplasts, since the distal blepharoplast has a faint fibril extending anteriorly from it, which may be the lower part of the fibril from the rod.

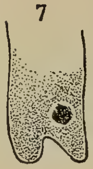
Rhodes (1926) finds similar rods (three or more) in *Heteronema acus,* which support the mouth and assist in food-getting. He says that Calkins is mistaken in calling the " staborgan " a parabasal body; that the " staborgan " is a true cytostome, and that Calkins is unjustified in creating the family, Heteronemidæ. *Peranema,* however, has but two rod-organs, and they appear to be morphologically similar. Neither of them has been observed to be contractile, as Rhodes says of the falcate rod in *Heteronema.* The writer has not observed that the rods of *Peranema* are of use in the ingestion of food, for while they are moved about in the metabolic activities that accompany ingestion, they remain close together, while the mouth stretches. Brown, however, has observed such a use. Schaeffer (1918) described " several " rods in *Jenningsia,* and they were in close relation to the mouth; he does not know of the use of them in feeding, although, by compression of the animal under a coverslip, he could force three of them out into the mouth.

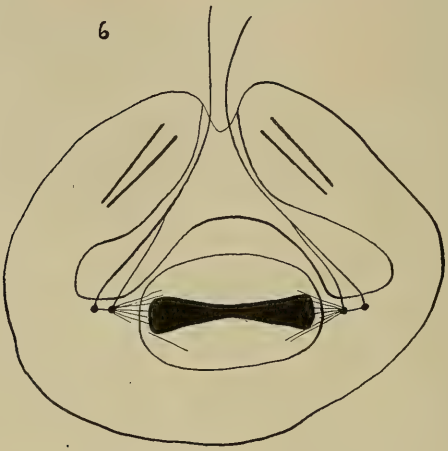
To justify the use of the term parabasal as applying to these rods, there should be traced a connection between them and the blepharoplasts. To do this beyond the possibility of a doubt has been impossible so far, but the case seems practically proved, by means of the fibrils referred to (Fig. 1). Dangeard (p. 291) regarded them as probably analagous to the tube in *Entosiphon sulcatus,* as does Brown. He thought they were probably united by " une pièce en forme de fer à cheval." This horseshoe-shaped piece he shows to be in the same location as the cytostomal element described by Hall, and the fibril the writer has endeavored to trace to the blepharoplasts, and while the tube of *Entosiphon* has three thickenings, it is not connected to the blepharoplasts. Calkins (p. 91) has a figure of *Peranema* which does not wholly agree with the findings of the writer, but which shows the connection clearly; if the two parabasals are in side view they would appear as figured by him. Lemmermann shows a connection between the rods and the basal granule of the flagellum. A similar connection is very clearly shown in his figure of *Urceolus cyclostomus* (Stein) Mereschk. Schaeffer (1918) shows a similar fibril from the anterior end of one rod proceeding towards the base of the flagellum in *Jenningsia.* Parabasals are generally said to originate from the nuclear material. Hall and Powell do not positively give the origin of the rods in their work on *Peranema,* nor do they claim the rods to be parabasals. In a large number of division figures studied by the writer, the old rods are seen to fade out gradually and new ones are formed *de novo,* in the region between the pole of the dividing nucleus and the new mouth (Fig. 3), which is in accord with Brown's findings. There is no evidence whatever of a nuclear origin nor of an origin from the blepharoplasts. Such knowledge would not justify calling them parabasals, even if they are connected to the blepharoplasts. Rather they may be looked upon as organelles of the mouth region, similar to those in *Jenningsia* and *Heteronema,* possibly used in feeding.

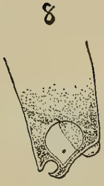
The posterior end of the animal often presents an amœboid appearance. At times a clear vacuole may be observed there, as in Fig. 8, but more often vacuoles filled with foreign matter are seen. These occasionally liberate their contents directly through the posterior end of the animal. Changes of shape in the posterior end are too slow to be called amœboid, and the region is best spoken of as a defecatory area, perhaps similar to that in *Anisonema.* What modification of the pellicle exists here is not known.

PLATE II









EXPLANATION OF PLATE II

FIG. 5. Diagram of the structure of a dividing *Peranema* according to Hall and Powell and reconstructed by the writer from a study of their work. The flagellum is pictured as single and the second blepharoplast of the writer is interpreted by them as a centrosome.

FIG. 6. Diagram of the structure of a dividing *Peranema* according to Brown and reconstructed by the writer from a study of his work. The flagellum is pictured as single but bifurcated at its base, and each of the two roots ends in a blepharoplast, the nearer one at each pole of the dividing nucleus serving as a centrosome or centriole.

FIG. 7. Posterior end of *Peranema* showing a vacuole containing matter for defecation. Drawn from life.

FIG. 8. Posterior end of *Peranema* showing a clear defecatory vacuole. Drawn from life.

Nutrition is certainly holozoic at times. Hall and Powell have described the formation of food vacuoles. In a culture which contains *Entosiphon,* these latter are often swallowed; as many as three have been observed distorting the shape of a *Peranema.* Often there are food vacuoles in the mid-region of the animal, which contain objects resembling bacteria, and ingested diatoms have been seen. But in old cultures the animals will become clear and show no food vacuoles; from which it may be inferred that saprophytic nutrition is possible. The statement of Brown that Euglenoida are necessary for food is incorrect. Perhaps the correct nutritive condition is an heterotrophic one.

The above characteristics seem to the writer to be diagnostic. They warrant taking the genus out of the family Astasiidæ, and transferring it to the family Heteronemidæ. They afford further justification for dropping the Peranemidæ as a family, as Calkins has done. Neither nutrition nor symmetry present sharp enough lines of demarcation to divide the order into families; furthermore, the nutrition of the colorless members of the order is too imperfectly understood and too subject to change. Above all, the studies show that until each member of this group is carefully investigated, their classification and relationships cannot be regarded as fixed.

SUMMARY

1. In addition to the usually described diagnostic features, *Peranema trichophorum* shows other and undescribed features as follows:

A. The ventral mouth begins as a short straight trough, then widens into a backward curving crescent.

B. There is an additional trailing flagellum emerging from the posterior part of the mouth and passing back beneath the body.

C. There are two rods of unknown function alongside the reservoir-gullet.

D. The blepharoplasts serve as division centers.

E. The posterior end of the body is a defecatory area.

2. The possession of the trailing flagellum transfers *Peranema* from the Astasiidæ to the Heteronemidæ, and justifies the reclassification of Calkins, whereby the family Peranemidæ was dropped.

LITERATURE CITED

BROWN, VIRGINIUS E., 1930. The Cytology and Binary Fission of Peranema. *Quart. Jour. Micr. Sci.* N. S., **73:** 403.

BÜTSCHLI, O., 1882–1888. Protozoa. In Bronn's Klass. u. Ordnung d. Thier-Reiche.

CALKINS, G. N., 1926. The Biology of the Protozoa. Lea and Febiger, Philadelphia.

CONN, H. W., AND C. H. EDMONDSON, 1918. Protozoa. In Ward and Whipple's "Fresh Water Biology."

DANGEARD, P. A., 1901. Recherches sur les Eugleniens. Le Botaniste. Série VIII.

DOFLEIN, F., 1916. Lehrbuch der Protozoenkunde. Fourth Edition. Gustav Fischer, Jena.

DUJARDIN, F., 1841. Histoire naturelle des zoophytes. Infusoires.

EHRENBERG, C. G., 1838. Die Infusionsthiere als Volkommene Organismen. Leipsic.

FISCH, C., 1885. Untersuchungen über einige Flagellaten und werwandte Organismen. *Zeitschr. f. wiss. Zool.*, **42**: 47.

HALL, R. P., 1926. Kinetic Elements and Nucleus in the Family Astasiidæ of the Euglenoid Flagellates. (Author's abstract.) *Anat. Rec.*, **34**: 155.

HALL, R. P., AND W. N. POWELL, 1928. Morphology and Binary Fission of Peranema trichophorum (Ehrbg.). *Biol. Bull.*, **54**: 36.

HARTMANN, M., AND C. CHAGAS, 1910. Flagellatenstudien. *Mem. Inst. Osw. Cruz*, vol. 2.

KLEBS, G., 1883. Über die Organization einiger Flagellatengruppen und ihre Bezeihungen zu Algen und Infusorien. *Unters. Bot. Inst. Tubingen*, vol. 50.

KLEBS, G., 1892. Flagellatenstudien. Theil I. *Zeitschr. f. wiss. Zool.*, **55**: 265.

LACKEY, J. B., 1929. Studies in the Life Histories of Euglenida.

I. The cytology of Entosiphon sulcatum (Duj) Stein. *Arch. f. Protist.*, **66**: 176.

II. The Life cycles of Entosiphon sulcatum (Duj) Stein, and Peranema trichophorum (Ehrenberg). *Arch. f. Protist.*, **67**: 128.

LEMMERMANN, E., 1913. In Pascher u. Lemmermann's Die Süsswasser-Flora Deutschlands, Osterreichs und der Schweiz. No. 2. Flagellatæ. 2. Gustav Fischer, Jena.

RHODES, R. C., 1926. Mouth and Feeding Habits of Heteronema acus. (Author's Abs.) *Anat. Rec.*, **34**: 152.

SCHAEFFER, A. A., 1918. A New and Remarkable Diatom-Eating Flagellate, Jenningsia diatomophaga, nov. gen., nov. spec. *Trans. Am. Mic. Soc.*, **37**: 177.

SENN, G., 1900. Flagellatenstudien. In Engler und Prantl's Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflangen. Vol. 1.

STEIN, FR., 1878. Der Organismus der Infusionsthiere.

WALTON, L. B., 1915. A Review of the Described Species of the Order Euglenoidina Bloch. Ohio Biological Survey, Vol. 1.

ONE STEP IN THE DEVELOPMENT OF HEREDITARY PIGMENTATION IN THE FISH ORYZIAS LATIPES[1]

H. B. GOODRICH

(*From Wesleyan University and the Marine Biological Laboratory*)

Studies by Aida (1921 and 1930) on the inheritance of the fresh-water fish *Oryzias* (*Aplocheilus*) *latipes* have shown that the various color effects may be referred to the action of genes as indicated by the following formulae:

Brown (wild type)	*B B R R*
Blue	*B B r r*
Red	*b b R R*
White	*b b r r*
Variegated on red	*B′ B′ R R*
Variegated on white	*B′ B′ r r*

The genes *B, B′,* and *b* control multiple allelomorphs and are autosomal and *R* and *r* are sex-linked. The development of these characters was studied by Goodrich (1927) with special reference to the effects controlled by genes *B, B′,* and *b*. The gene *B,* which is dominant to both *B′* and *b,* permits the maximum development of the melanophores which become uniformly distributed over the body surface. *B′* is dominant to *b* and causes variegation. In this case there are two types of melanophores present. One form is dark colored, having the normal development of melanin, and is irregularly distributed, producing the mottled appearance of the fish. The other type is found in areas not occupied by the darker form and it contains so little melanin that it is usually invisible. It may be detected, however, by treatment with adrenalin (Goodrich, 1927) or by other methods which will induce a concentration of the previously diffusely distributed melanin and thus render the cells visible. In the recessive condition (gene *b*) very few of the deeply pigmented melanophores can be found. Practically all are of the light or "Farblose" (Schuberg, 1903) variety.

In attempting to analyze this situation the question may first be raised as to whether intracellular or extracellular factors are critical in determining which type of melanophore will be developed. It has been previously pointed out (Goodrich, 1927) that an extracellular factor such as a circulating hormone seems improbable. Such a hormone would be nearly uniformly distributed throughout the body and similar cells would be similarly affected. This then, acting alone, could not be the critical factor in the production of a variegated pattern. It seems therefore reasonable to suppose that the differential factor lies within the cells.

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[1] This study is part of a program dealing with the development of Mendelian characters and color patterns in fish which is made possible by grants from the Denison Foundation for Biological Research at Wesleyan University. The writer desires to acknowledge the assistance given by C. B. Crampton and Rowena Nichols.

As the two types of melanophores apparently differ only in the amount of pigment produced the factors involved in melanin production have been investigated. For this purpose the so-called dopa reaction (Bloch, 1917) has been utilized. It is well known that tyrosine is the chromogen which is the precursor of melanin. Dopa is 3 : 4 dihydroxyphenylalanine, which is considered to be the first transformation product in the change from tyrosine to melanin. It therefore may be used as a test for the presence of such an oxidase. For further details of the chemistry of the dopa reaction Raper (1928) may be consulted.

In these experiments scales have been removed from the yellow (red) fish and from light areas in variegated fish and treated with dioxyphenylalanin.[2] The methods used were those outlined by Laidlaw (1932). The method is simple and the results clear. In from two to three hours after placing the fresh scales in a dopa solution there may be seen in each scale a few cells which are amœboid in form and were previously nearly invisible but have become densely blackened. Other types of tissue cells do not show this reaction. This transformation is shown in Figs. 1 and 2. These are microphotographs taken with a yellow light filter which is necessary in order to eliminate the xanthophores which would otherwise be almost indistinguishable in the photograph from the melanophores. Figure 1 shows a fresh scale from a yellow fish. The light colored melanophores have contracted slightly and are therefore faintly visible, but can in this picture be only surely identified by careful comparison with Fig. 2 which shows the same scale after treatment with dopa.

These results then seem to indicate that the " colorless " chromatophores and no other cells contain an enzyme, the dopa oxydase (Bloch, 1917), which is probably identical with tyrosinase and which is necessary for the formation of melanin. These cells were, however, lacking in a precursor of melanin and the treatment described above has substituted dopa for that precursor.

It seems, therefore, probable that during histogenesis both types of melanoblasts possess the necessary oxydase but in one—that one which

[2] The dioxyphenylalanin was obtained from the Hoffman La Roche Co.

is to produce the "colorless" melanophore—there exists an insufficient quantity of chromogen. The three phenotypes are then probably similar in the production of oxydase but differ in chromogen content. The chromogen is present in maximum amount in all chromatophores of the dark form (gene *B*), is present in minimal amount in all chromatophores of the light types (gene *b*), and in the variegated types it is present in maximum amount in some cells and in minimal amount in other cells. This condition in the variegated types provides an added problem which apparently can be solved only in terms of some sort of embryonic segregation. It seems probable, as has been previously suggested (Goodrich, 1927), that at an earlier stage of development a segregation of mesenchymal anlagen for the prospective pigmented and non-pigmented melanophores may occur and the cells so differentiated may become variously distributed by a process of amœboid migration characteristic of this type of cell. Such a period of segregation would mark the latest stage in development at which the critical cytoplasmic determination could occur in the variegated type and by inference it is to be expected that the cytoplasmic determination in the uniformly colored types would occur at a corresponding stage.

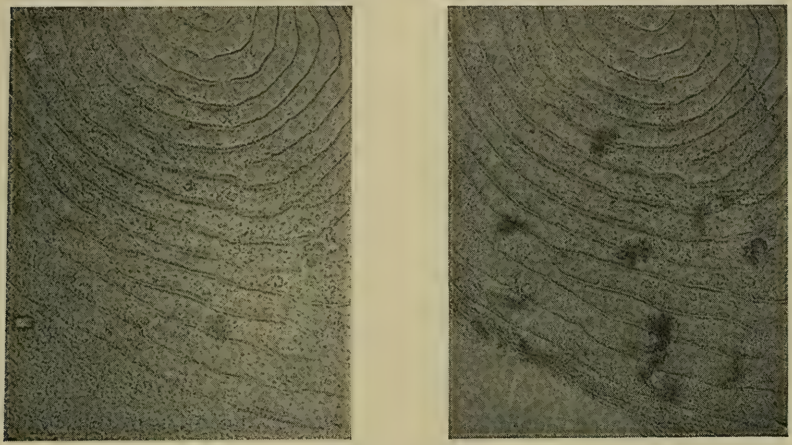

FIG. 1 (left). A microphotograph (× 120) of a part of a fresh scale from a yellow fish. The partially contracted light colored melanophores are almost invisible.

FIG. 2 (right). The same scale after treatment with dopa. Melanophores can now be readily located.

Dopa treatment has also been applied to the scales of the red and transparent forms of goldfish. In these fish (cf. Goodrich and Hansen, 1931) it is known that the melanophores disintegrate during an early growth period and no evidence has been advanced to show the presence of " colorless " melanophores. The dopa test on these scales gave an entirely negative result. This gives further support to previous conclusions that the light phases of these two different species have been produced by very different developmental processes.

CONCLUSIONS

The light colored recessive phenotypes of the fish *Oryzias latipes* have been shown by use of the dopa reaction to possess the necessary oxydase for the formation of melanin. It is inferred that these differ from the dark colored dominant phenotypes in the relative absence of the chromogen tyrosine. Both chromogen and oxydase first appear intracellularly in the melanoblasts.

This condition is contrasted with that found in the goldfish where negative results were obtained.

REFERENCES

AIDA, TATUO, 1921. On the Inheritance of Color in a Fresh-Water Fish, Aplocheilus latipes Temmick and Schlegel, with special Reference to Sex-Linked Inheritance. *Genetics*, **6**: 554.

AIDA, TATUO, 1930. Further Genetical Studies of Aplocheilus latipes. *Genetics*, **15**: 1.

BLOCH, BR, 1917. Chemische Untersuchungen über das spezifische pigment bildende Ferment der Haut, die Dopaoxydase. *Zeitschr. f. physiol. Chem.*, **98**: 226.

GOODRICH, H. B., 1927. A Study of the Development of Mendelian Characters in Oryzias latipes. *Jour. Exper. Zool.*, **49**: 261.

GOODRICH, H. B., AND I. B. HANSEN, 1931. The Postembryonic Development of Mendelian Characters in the Goldfish, Carassius auratus. *Jour. Exper. Zool.*, **59**: 337.

LAIDLAW, G. F., 1932. The Dopa Reaction in Normal Histology. *Anat. Rec.*, **53**: 399.

RAPER, H. S., 1928. The Aerobic Oxidases. *Physiol. Rev.*, **8**: 245.

SCHUBERG, A., 1903. Untersuchungen über Zellverbindungen. *Zeitschr. f. wiss. Zool.*, **74**: 155.

SCALE TRANSPLANTATION IN THE GOLDFISH CARASSIUS AURATUS[1]

I. Effects on Chromatophores. II. Tissue Reactions.

H. B. GOODRICH AND ROWENA NICHOLS

(From Wesleyan University and the Marine Biological Laboratory)

Materials and Methods

The existence of abundant variation in color and pattern in the goldfish, *Carassius auratus,* and the fact that the mode of color inheritance has been determined (Chen, 1928 and Berndt, 1928) suggested this fish as desirable material for a study of the development of inherited color patterns and the origin of pigment cells. Certain phases of the early development of the color pattern were studied by Goodrich and Hansen (1931). It was then felt that some information in regard to later phases of histogenesis and about the necessary tissue requirements of the different chromatophores might be obtained by a study of tissue transplants. The following five types of goldfish have been used for this purpose:

Brown or wild type of American breeders (normal-scaled)
Gold (normal-scaled)
Gold and silver (normal-scaled)
Transparent Shubunkin (transparent-scaled)
Ordinary Shubunkin or Calico Fish (designated "mottled" by Chen; largely transparent-scaled).

Chen (1928) describes the normal-scaled condition in goldfish as one in which all scales are backed with a layer of reflecting tissue. According to this classification the first three of the above varieties are normal-scaled. The types, gold, gold and silver (and the black and gold not used in these experiments) are also described by Fukui (1927) and Berndt (1928) as arising by a process of progressive depigmentation from the self-colored brown of the young fish (see Goodrich and Hansen, 1931). It seems clear that there occurs a permanent block to the process of depigmentation at different points in individual fishes.

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[1] This study is part of a program, dealing with the development of Mendelian characters and color patterns in fish, which is made possible by grants from the Denison Foundation for Biological Research at Wesleyan University.

The cause of this block, whether hereditary or due to other factors, is at present unknown. It has, however, been shown (Chen, 1928 and Berndt, 1928) that the mottled fish or ordinary Shubunkin is a heterozygous type from the cross between the transparent Shubunkin and, apparently, any type having normal scales. Goodrich and Hansen (1931) have shown that the mottled and transparent types also arise by a process of depigmentation which begins much earlier in ontogeny than is the case with the first three named varieties.

The brown goldfish is a dark olive drab which grades ventrally to a silvery gray. All the scales are backed with reflecting tissue consisting of guanin crystals, and the dermis is abundantly pigmented with melanophores and xanthophores which together give a dull brown. The gold variety is likewise normal-scaled and the presence of numerous xanthophores in the dermis gives them their characteristic brightness. Great variation in color pattern ranging from gold with small silver areas to predominantly silver with small patches of gold exists in the gold and silver fish. Scales in the silver areas are without chromatophores and are therefore white in appearance, but the reflecting tissue shining through the scales gives a decided luster best described as silvery.

The transparent Shubunkin is largely without reflecting tissue. Absence of the guanin crystal layer renders the scales and tissues transparent, which undoubtedly led fish fanciers to give this form the name "scaleless." In this type we find marked variation in the amount and kinds of pigment. Sometimes chromatophores are entirely lacking and the individual is white, but more frequently there are a few deep-lying melanophores on the dorsal side of the body and in the region of the caudal peduncle. An occasional scale is backed with reflecting tissue and infrequently a transparent fish has a small area of xanthophores on the body or head.

The color pattern of the mottled fish or ordinary Shubunkin is much more complex than that of any of the fish already described. There is no uniformity in arrangement of colored scales nor those backed with reflecting tissue. A variegated design of black, yellow, and white, with a bright spot or two of gold and silver, characterizes this fish. Chen (1928) and Goodrich and Hansen (1931) give a more detailed description of goldfish varieties and an account of the variation of color pattern occurring in these forms.

The operation of transplanting tissue is very simple owing to the fact that the exposed part of the scale is covered with pigment-bearing dermis and epidermis, and removal of the scales carrying this tissue is easy. Transplantations of the skin of fish have been made by Šećerov

(1912). This method is probably impractical in goldfish and is much less convenient than the method of scale transplantation which seems to have first been used by Mori (1931). When removed, each scale leaves a small pocket in the dermis which facilitates the introduction of another scale, thus eliminating the necessity of an artificial means of attaching the transplanted tissue. Scales were removed from the host fish by means of a pair of fine forceps and the scales from the donor previously removed were tucked into the empty spaces. If more than a minute was to elapse between the removal of a scale and transplantation, the scale was kept in normal salt solution. Frequently there was a discrepancy between the size of scales exchanged and therefore the pockets they filled. It was found that by trimming the base of the scale to the desired size the edges of host and donor tissue could be brought into close proximity, thus insuring more successful healing of the transplant without any apparent injurious effects. For this operation and later observations on the scales the fish were anaesthetized with chloretone. A coördinate system utilizing the lateral line as a base was devised for locating all transplanted scales.

I. Effects on Chromatophores

Autoplastic Transplantation

Autoplastic transplantations, or transplantations to another part of the same individual, proved equally successful on all varieties of goldfish. Within forty-eight hours circulation was established on the scale and frequently the transplant had a functioning capillary net-work within thirty hours after the operation. In the case of autoplastic transplantation of pigmented scales a temporary contraction of the chromatophores occurred. Soon after the circulation was established, however, the cells were observed to be in the same condition as those on the rest of the body. This temporary contracted state is probably due to the failure of the cells to receive nervous impulses after the severing of the tissue (cf. review by Parker, 1930). Further investigation would be necessary to determine whether the contracted condition of melanophores on dark scales after transplantation approximates the stellate condition of denervated melanophores of *Fundulus* reported by Wyman (1924). There is no loss or change in the reflecting tissue when a scale is put into a new position on the same fish. In homoiotransplantations and those between different varieties of goldfish the reflecting tissue degenerates rapidly. Therefore this particular type of tissue serves as a very sensitive indicator of partial or complete success of a transplantation.

No degeneration of chromatophores or of reflecting tissue occurs in autoplastic transplantation in uniformly colored fish or in any other type except in young fish undergoing depigmentation. Other results, however, obtained from transplantations on the mottled Shubunkin and the gold and silver type have bearing on the problem of pattern formation.

In the mottled fish a black scale placed in either a white or gold region retains its melanophores. A light scale (white or gold) placed in a dark region assumes a complete complement of melanophores and often also of xanthophores, if they are present in the dark area. Melanophores first appear at the periphery and later invade the center of the scale, indicating a migration rather than a development in situ. Similarly, a gold scale placed in a white region retains its pigment and a white scale becomes invaded by xanthophores in a gold region. The migration of xanthophores is markedly slower than that of melanophores. In the ordinary Shubunkin after about ten days a light scale transplanted to a dark area has a complement of melanophores, whereas on a gold and silver fish approximately twenty-five days are required for a silver scale to become completely gold. The proliferation and migration of chromatophores varies markedly between individuals according to age and other undetermined factors; therefore, the above estimates of time are averages. A summary of the experiments is shown in Table I.

It has also been found that chromatophores will migrate from a transplanted scale to the surrounding region. Here again the activity of the melanophores is more pronounced than that of the xanthophores. Of 35 dark scales put into light areas on the mottled type of fish, 25 showed a spreading of melanophores sometimes to the extent of twice the diameter of the scale. The phenomenon occurs more frequently in scales transplanted into the lateral line, below it, or positions not more than two scale rows above it. The melanophores more frequently wander into the immediately sub-epithelial dermis but are sometimes found in the dermis lying beneath the scale.

The gold-silver fish proved more favorable than the mottled Shubunkin for testing the migration of xanthophores but the results in both cases are similar. Gold scales transplanted to light areas retained their color but xanthophores rarely migrated until after four months in the new location. After this time xanthophores were observed in the tissue surrounding gold scales but they had migrated not more than about one-fifth of the diameter of a scale.

In order to test the behavior of tissue that has recently lost a complement of melanophores, transplantations were made on young goldfish undergoing depigmentation. These fish are black and gold, the black area covering the dorsal aspect of the body. Fukui (1927) described the progress of depigmentation in the common goldfish as a wave of melanophore destruction that starts in the belly region and progresses dorsally. For further discussion of depigmentation of goldfish varieties, see Goodrich and Hansen (1931). Gold scales from the belly region or lower sides were exchanged with the more dorsal black scales. There was rapid healing of the tissue and the black scales lost their melanophores within two or three days. In two cases the fish turned gold so rapidly that no pigment change occurred in the gold scales put into the dark region. In four fish, however, the process of depigmentation was slower and fifteen gold scales put into the dark region of these fish were completely covered with melanophores from five to seventeen days after the operation. Frequently, as one might expect, these scales lost the newly acquired melanophores within a few days, for the process of depigmentation overtook them even in their new position.

TABLE I

Autotransplants

Type of Fish	No. of Scales	Type of Transplant	Fate of Chromatophores
Gold..............	30	Gold on gold	No change in chromatophores.
Brown.............	32	Black on black	No change in chromatophores.
Shubunkin..........	41	White or gold on black	Assumed complement of melanophores.
Shubunkin..........	35	Black on white or gold	Kept melanophores, and in case of 25 scales there was a spreading of melanophores into the surrounding dermis.
Gold and silver......	46	Gold on silver	Kept xanthophores, and in all cases there was a slight migration of these cells to the surrounding dermis.
Gold and silver	26	Silver on gold	Assumed complement of xanthophores.
Young goldfish (in depigmentation).....	17	Black on gold	Lost all melanophores.
Young goldfish (in depigmentation).....	20	Gold on black	17 assumed complement of melanophores.*

* On fish kept in cold room melanophores did not invade gold scales.

In order to retard depigmentation one fish was kept in a cold room.

Only after sixty-eight days were melanophores observed on two gold scales transplanted to the black area. The lower temperature not only inhibited depigmentation but also retarded the migration of melanophores from the surrounding dark area into the light scales.

Homoiotransplants

In all cases of transplantation between different individuals of the same or different varieties the chromatophores were found to disintegrate. This was preceded by the disappearance of reflecting tissue. In cases where the scales were placed in the self-colored types (wild or gold) they usually later assumed the complete host pigmentation. This is known to have occurred in 270 out of 343 successful transplants. In cases of transplantations into Shubunkins the results varied according to the pigmentation of the region that received the scale.

A complete tabulation and further details of these results will be given in Part II.

Discussion

The work of Chen and Berndt (*loc. cit.*) has indicated that the mottled type is the hybrid between the transparent Shubunkin and the "scaled" forms which include the brown, gold, gold and silver varieties in these experiments and which are therefore genetically similar in regard to the characteristic "scaled." The hybrid contains melanophores irregularly distributed and these may be practically absent from the adults of either homozygous parent. These experiments show that melanophores may live and multiply over any part of the surface of the body of the hybrid. Knowledge of the process of depigmentation (Fukui, 1927; Goodrich and Hansen, 1931) indicates that melanophores cannot exist for long periods in most adult goldfish. Melanophores transplanted from the diminishing black areas to gold areas disintegrated at once. The work of Goodrich and Hansen (1931) on the development of the transparent type also shows that in those forms some condition is present which destroys melanophores. Unfortunately, the existence of tissue antagonism (Part II), which prevents the successful transplanting of melanophores from one fish to another, does not permit a more direct analysis of the reaction of the homozygous types to melanophores.

The inference, however, seems fairly clear that while growth and persistence of melanophores is possible in the heterozygous type, some inhibition exists in the white homozygous form and sometimes (the complete gold type) in the other homozygous parent. The work of Smith (1932) has shown that melanin formation may be induced in gold types by the use of X-rays or by extensive de-scaling (Smith, 1931), both of which treatments may be considered as tissue irritants. The melanophores so produced, however, do not persist, which again indicates that tissue or physiological conditions are not favorable for them in these types. Unpublished experiments with X-ray treatment by Goodrich indicate that the transparent fish has less power to form melanophores than the gold type. Xanthophores, on the other hand, may be present in all types but in decreasing abundance as we pass from the scaled to the hybrid to the transparent forms. When, however, melanophores are in a favorable environment, they are far more active than xanthophores. These results are in agreement with the findings of Reis (1926) and Bock (1926) on Amphibia.

II. Tissue Reactions, Homoiotransplants

More than one thousand transplantations of tissue-bearing scales have been made between different fishes. The scale is imbedded in the dermis which normally contains all of the chromatophores and this is covered superficially by the non-pigmented epidermis. The transplant then consists of the scale and dermis and epidermis. In all cases the homoiotransplants have shown some degree of incompatibility or antagonism with the host tissues. In no case whatever have the donor chromatophores or the guanin crystals persisted. These have disintegrated and have been replaced by new chromatophores and guanin crystals if these were characteristic of the host. All possible exchanges between the four types of fish have been made, including transplants between fish of the same types.

Practically all of our fish have been obtained from one fish breeder,[2] and it is therefore probable that some of the transplants have been between closely related individuals (syngenesiotransplants in the terminology of Loeb, 1930). According to Loeb, less antagonism would be expected in such cases. In order to investigate the possibility that some of our fish might be so closely related that no antagonism would arise, transplants were made on a number of occasions between one gold type and five or six different gold type fish. Little of the extreme type of antagonism was noted, but in all cases the donor chromatophores disintegrated. Evidence in regard to the persistence of other tissues is discussed later.

The disappearance of chromatophores has, however, been the only constant feature of the homoiotransplants. There has been much variation in the rate of disintegration of the chromatophores, in the persistence of other dermal tissues, including the horny scale, and possibly in the persistence of the surface epithelium. A decided inflammation frequently occurs shortly after transplantation. None of these reactions has appeared in autotransplants made under conditions that were otherwise identical. The inflammation, if it occurs, usually arises within three days after transplantation and disintegration of chromatophores follows shortly. If there is no inflammation, the destruction of the chromatophores may be delayed until from ten to fourteen days after transplantation, which is also about the usual time of reëstablishment of the circulation, while in autotransplants the circulation may be established within thirty hours. New pigment cells appear at this time at the edge of the scales. The keratinous scales may be dissolved after four or five weeks. Observations have been continued for two or three months if transplants were not completely incorporated earlier.

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[2] The fish have been obtained from the Grassyfork Fisheries, Inc., of Martinsville, Indiana. We are indebted to this firm for very careful attention to our orders and requests.

Microscopic sections show a striking contrast between autotransplants and homoiotransplants. The former differ in no way from sections of normal untransplanted scales while the latter show a progressive thinning of the epidermis and a disintegration of the dermis. The chromatophores are the first cells observed disintegrating. Melanin granules pass to the surface epithelium and are sloughed off. Abundant leucocytes can be observed in fresh tissue. No clearcut edge of advancing host tissue such as that described by Rand and Pierce (1932) has been observed.

Homoiotransplants have been classified as follows on the basis of the various types of reactions: (1) transplants in which the donor scale is retained, (2) transplants in which the keratinous scale is dissolved to be later replaced by regeneration. This second group is further subdivided into those which show inflammation of the dermis and those which do not become inflamed. Inasmuch as no autotransplants have shown inflammation, it has been felt that this was the result of tissue incompatibility and not due to faulty technique. The donor chromatophores disintegrate in both classes. It is usually impossible to determine the fate of other dermal tissues.

Transplants are usually made by reciprocal exchange between pairs of fish and from three to five such exchanges being made per pair. The fate of the transplants on a given fish has been essentially the same. Hence it is obvious that it is a physiological reaction of the individual fish which is tested—not that merely of a given transplant. The results reported here are based on two years' work (1931–32) and on two separate lots of fish. The first lot included 840 transplants and the second 253 transplants. The second series was undertaken chiefly to fill in certain gaps in the first series. The results here emphasized are, however, based chiefly on the first and larger series (Table II) and are limited to those transplants of which it has been possible to keep a complete record from start to finish. This reduces the number in the first series to 695. The second series, Table III, differs from the first in being less complete, as certain classes are omitted; there were fewer cases of inflammation and a much larger record of scales dissolved. This may have been due to more careful attention given to this feature or the second lot may have come from a more inbred stock. If all the percentages in Table II are increased by an equal amount, some degree of correlation will be noted between the two tables except in the case of the transplants between transparent and transparent. Observations on transparent fish are especially difficult and liable to error. It is felt, however, that the conditions of observation in the first lot were more uniform and that it is not legitimate to combine the results of both years' work in a single table. Sex of goldfish cannot be determined by external inspection except during the breeding season. Our records give no indication of a sex-differential in the reactions of the transplants.

TABLE II

Homoiotransplants. First series 1931–32.

Transplants	Number of scales	Scale dissolved		Scale incorporated	Percentage dissolved
		Rapid reaction Inflammation	Slow reaction No inflammation		
					per cent
Brown to brown	22	0	0	22	0
Brown to gold	18	0	0	18	0
Brown to Shu.	15	0	0	15	0
Brown to trans.	18	0	14	4	77.8
Gold to gold	85	9	0	76	10.6
Gold to brown	30	0	0	30	0
Gold to Shu.	30	5	2	23	23.3
Gold to trans.	55	5	8	42	23.6
Shu. to Shu.	29	3	20	6	79.3
Shu. to brown	13	1	0	12	7.7
Shu. to gold	24	4	0	20	16.6
Shu. to trans.	23	11	3	9	60.9
Trans. to trans.	51	12	19	20*	60.8
Trans. to brown	67	0	0	67	0
Trans. to gold	102	9	14	79	22.5
Trans. to Shu.	113	61	9	43	61.9

Shu. = shubunkin.
Trans. = transparent.
* 15 of these scales recovered in spite of severe inflammation.

Discussion

The fact that all homoiotransplants in goldfish show tissue incompatibility while autotransplants show no antagonism establishes the existence in fish of individuality differentials in the sense in which this term is used by Loeb. This class of vertebrates may then be removed from the list given by Loeb (1930), p. 583, which shows no individuality differentials.

If the percentages of occurrence of transplants in which the scales dissolved as indicated in Table II are rearranged (Table IV), certain relationships become apparent. It appears that with the exception of the transplants from brown to transparent these types of reactions are much less likely to occur when the brown form is either donor or host than in any other exchanges. It may be noted that the high figure given for transplants from brown to transparent is not substantiated in the second series and that no cases of inflammation were recorded. The type here referred to as "brown" is the dark colored form occasionally appearing in breeders' stocks which most closely resembles the true wild fish. It is highly probable that the other types have arisen by mutation and by hybridization from the wild stock. It then appears that transplants to or from the wild-like form are more likely to be successful than those between mutant types.

TABLE III

Homoiotransplants. Second series 1932–33.

Transplants	Number of scales	Scale dissolved		Scale incorporated	Percentage dissolved
		Rapid reaction Inflammation	Slow reaction No inflammation		
					per cent
Brown to brown	26	0	11	15	42.3
Brown to gold	30	0	8	22	26.7
Brown to Shu.	32	0	10	22	31.2
Brown to trans.	29	0	8	21	27.6
Gold to brown	23	5	11	7	69.6
Gold to Shu.	10	5	5	0	100.0
Shu. to Shu.	25	0	25	0	100.0
Shu. to brown	27	4	12	11	59.2
Shu. to gold	12	0	4	8	33.3
Shu. to trans.	10	0	10	10	100.0

According to Chen (1928) the brown type is, in regard to the characters which he studied, to be considered as genetically identical with the gold type. It is probable, however, that most Shubunkins are produced by crosses between the transparent and the gold types. It is therefore of some interest to note the relations existing between the gold and transparent types and their hybrid, the Shubunkin. These are shown in the portion of Table IV enclosed by the heavier line. It will be seen that the transplants involving the gold type are more successful than those limited to the white and transparent forms. The whole relationship seems to suggest that the further removed a type is from the characteristics of the wild stem form, the nearer it is to a condition of unbalance in its tissue relations and that this condition is revealed in transplants. This situation may possibly also be correlated with the conditions of greater variability in development in the transparent form and the Shubunkin when contrasted with the gold type as shown by Goodrich and Hansen (1931).

TABLE IV

Percentage of homoiotransplants from first series showing destruction of scales. (Scale dissolved).

Donor	Host			
	Brown	Gold	Shu.	Trans.
Brown	0	0	0	78
Gold	0	11	23	24
Shu.	8	17	79	61
Trans.	0	22	62	61

Loeb (1930) has emphasized the point that grafts from a parent strain to a hybrid show relatively slight antagonism, while a marked reaction is likely to occur when a transplant is made in the reverse direction. It will be noted that the results from the goldfish are not in accord with this generalization.

Autotransplants of skin have been found in various groups of animals to be generally successful and to show relatively little tissue antagonism (cf. review by Korschelt, 1931). In many cases there is evidence of an interpenetration of tissues between the graft and the host until an equilibrium is reached. This does not usually appear to involve any extensive destruction of tissues, but the results of Rand and Pierce (1932) on Amphibia indicate that caution is necessary in drawing conclusions from total preparations. The results here outlined in Part I from autotransplants, especially in the Shubunkin and in the gold and silver type, give evidence of the interpenetration of tissues. This is shown by the migration of the chromatophores and closely parallels such results as those obtained by Sale (1913) and Seelig (1913) on guinea pigs. Šečerov (1912) found a less rapid penetration in autotransplants of the skin of the fish *Nemachilus barbatula* and a relatively slow or incomplete replacement of tissue in homoiotransplants.

CONCLUSIONS

Part I

1. Autotransplants on all types of goldfish succeeded without loss of any of the tissue elements, epithelium, dermis, or horny material.

2. Autotransplants on fish having two or more different colored areas showed that scales without pigment when put into either gold or black regions assume a complement of chromatophores similar to the surrounding area. Pigmented scales keep their chromatophores when put into an unpigmented region and there is a tendency for these pigment cells to invade the surrounding dermis. Melanophores were found to migrate faster than xanthophores and to wander farther from the graft.

3. In homoiotransplants of scales between individuals of the same or different varieties, the reflecting tissue and chromatophores degenerated. After the loss of donor pigment, however, many of the scales assumed the chromatophores of the host.

Part II

4. Some degree of tissue incompatibility is found in all homoiotransplants. This varies from that apparently causing only the disintegration of chromatophores to that bringing about the destruction of the whole scale and accompanied by inflammation.

5. A wide variation in degree of tissue antagonism has been observed even between similar pairs of fish. The following relations, however, seem to be indicated:

A higher percentage of tissue antagonism is usually observed when the less pigmented types (Shubunkin and transparent) are involved than when the more pigmented types (brown and gold) are used, and transplants with the brown type show less antagonism than any other exchanges.

The further removed a pair of individuals is from the characteristics of the wild form, the more likely it is that incompatibility will be found in transplants between them.

REFERENCES

Berndt, Wilhelm, 1928. Wildform und Zierrassen bei der Karausche. *Zool. Jahrb.*, **45**: 841.

Bock, Rosa, 1926. Sur le comportement des greffes de la peau des Amphibiens. Greffes homoplastiques de la peau des Salamandres adultes. *Compt. rend. Soc. Biol.*, **95**: 506.

Chen, Shisan C., 1928. Transparences and Mottling, A Case of Mendelian Inheritance in the Goldfish, Carassius auratus. *Genetics*, **13**: 434.

Fukui, Kenichi, 1927. On the Color Pattern Produced by Various Agents in the Goldfish. *Folia. Anat. Japon.*, **5**: 257.

Goodrich, H. B., and I. B. Hansen, 1931. The Postembryonic Development of Mendelian Characters in the Goldfish, Carassius auratus. *Jour. Exper. Zool.*, **59**: 337.

Korschelt, E., 1931. Regeneration und Transplantation. Berlin.

Loeb, Leo, 1930. Transplantation and Individuality. *Physiol. Rev.*, **10**: 547.

Mori, Yasumasa, 1931. On the Transformation of Ordinary Scales into Lateral Line Scales in the Goldfish. *Jour. Fac. Sci. Imp. Univ. of Tokyo*, **2**: 185.

Parker, G. H., 1930. Chromatophores. *Biol. Rev.*, **5**: 59.

Rand, Herbert W., and Madelene E. Pierce, 1932. Skin Grafting in Frog Tadpoles: Local specificity of skin and behavior of epidermis. *Jour. Exper. Zool.*, **62**: 125.

Reis, Karoline, 1926. Sur le comportement des greffes de la peau des Amphibiens. Métamorphose des Greffes de la peau larvaire sur les Salamandres adultes. *Compt. rend. Soc. Biol.*, **94**: 349.

Sale, Llewellyn, 1913. Contributions to the Analysis of Tissue Growth. VIII. Autoplastic and homeoplastic transplantation of pigmented skin in guinea pigs. *Arch. f. entw.-mech.*, **37**: 248.

Šečerov, Slavko, 1912. Weitere Farbwechsel-und Hauttransplantationversuche an der Bartgrundel (Nemachilus barbatula L.). *Arch. f. entw.-mech.*, **33**: 716.

Seelig, M. G., 1913. Contributions to the Analysis of Tissue Growth. IX. Homöoplastic and autoplastic transplantation of unpigmented skin in guinea pigs. *Arch. f. entw.-mech.*, **37**: 259.

Smith, G. M., 1931. Occurrence of Melanophores in Certain Experimental Wounds of the Goldfish (Carassius auratus). *Biol. Bull.*, **61**: 73.

Smith, G. M., 1932. Melanophores Induced by X-ray Compared with Those Existing in Patterns as Seen in Carassius auratus. *Biol. Bull.*, **63**: 484.

Wyman, L. C., 1924. Blood and Nerve as Controlling Agents in the Movements of Melanophores. *Jour. Exper. Zool.*, **39**: 73.

THE DIFFERING EFFECTS OF DIFFERENT PARTS OF THE VISUAL FIELD UPON THE CHROMATOPHORE RESPONSES OF FISHES

FRANCIS B. SUMNER

(From the Scripps Institution of Oceanography, University of California, La Jolla, California)

In two previous publications[1] I have discussed a problem which presents itself when we consider the chromatophore responses of fishes to their background, under various conditions of illumination. It has long been known that many fishes become extremely dark upon a black background and very pale upon a white one, while they assume intermediate shades upon backgrounds which are likewise intermediate. An interesting feature of this situation is that the response to a given background is largely independent of the absolute degree of illumination of the latter. A black background, in direct sunlight, will call forth the maximum degree of darkening, while a white background, in rather dim light, will call forth nearly or quite the maximum degree of pallor. In a gray container, too, the shade of the fish will approximate that of its surroundings, regardless of the intensity of illumination. It is obvious, however, that a neutral gray surface, brightly illuminated, may reflect far more light—specifically, far more *white* light—than a white surface dimly illuminated. Why then, does the fish assume a darker shade on the former than on the latter?

To answer this question, I believe, we must consider why we ourselves recognize a surface as black, white, or gray, regardless of the degree of illumination to which it is subjected. The answer is simple. The object in question is perceived in relation to the total visual field. When, on a dark night, we recognize a sheet of white paper as white, it is because we make a "correction," as it were, for the dimness of the general illumination. If, for any reason, we are misled respecting the degree of illumination to which an object is subjected, we may see a gray object as white, or vice-versa.

Does not the fish react in a manner quite similar to this? For flatfishes, I have already argued that the animal is controlled not by the absolute intensity of the light reflected from the substratum, but by the ratio between this and the source of illumination overhead.[2] In the more recent paper by Sumner and Keys (1929), it was shown experimentally that when the underlying surface was caused to be considerably lighter or darker than would normally result from the given apparent source of illumination, the fish became paler or darker, as the case might be, than the background in question. Similar experiments with catfish (Pearson, 1930) have confirmed these results, at least within a considerable range of illumination.[3]

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[1] Sumner, 1911; Sumner and Keys, 1929.

[2] Sumner, 1911. In this idea of reaction to a ratio, I was anticipated by Keeble and Gamble (1904).

Since, of course, the fish has no notion respecting the source of illumination nor any means of identifying this, we may say that it reacts to a ratio between light received from two different portions of the visual field. One of our present problems is to identify these two portions, if only approximately. How much of the total field belongs in the numerator of our fraction; how much in the denominator? How much acts in a positive sense, such that making it lighter or darker in relation to the rest causes the fish, likewise, to become lighter or darker; how much in a negative sense, such that increasing or decreasing its relative brightness calls forth converse changes on the part of the fish? While I have not been able to answer these questions in a quantitative way, I trust that some light is thrown upon the subject in the following pages.

In my earlier experiments with the small flatfish, *Rhomboidichthys* (=*Platophrys*) *podas* (Sumner, 1911), I showed that the bottom of the container in which the fish was kept was far more influential in determining the chromatophore responses of the latter than were its walls. This was surprizing, in view of the fact that the fish itself commonly covered a very considerable fraction of the bottom, and that it lay much of the time with its head fairly close to the vertical walls of the jar. In another flatfish, *Lophopsetta maculata,* while the influence of the bottom was predominant under ordinary conditions, that of the near-by side walls of a small container was much more pronounced than in *Rhomboidichthys.* This difference between the two species was due, perhaps, to the lesser degree of motility of the eyes of *Lophopsetta,* as compared with those of *Rhomboidichthys,* which are placed on movable stalks and enable the fish to look almost directly downward.

If the color changes of fishes have a bionomic value in relation to concealment, one would expect bottom-dwelling fishes such as flounders to be influenced primarily by the substratum on which they lie. In freely swimming fishes, on the other hand, which move at various levels through the water, one would not expect the substratum to exercise such a predominant influence.

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[3] Earlier experiments by Mast (1916), with somewhat different methods, had given results which only partially harmonized with those here cited. Mast's procedure has been criticized by Summer and Keys (1929).

I have performed several experiments upon the common Pacific killifish, *Fundulus parvipinnis* Girard, in order to determine the relative influence of the bottom and walls of the small glass aquaria in which the fishes were kept. Ten medium-sized specimens were placed in each of two shallow, cylindrical glass aquaria, 25 cm. in internal diameter by 14 cm. deep.[4] In one of these, the bottom was painted black, the walls white; in the other the condition was reversed. At the close of two months (the definitive condition was doubtless reached in much less time), the following conditions were noted. The eight survivors in the black-bottomed, white-walled aquarium were distinctly darker and more opaque than those in the white-bottomed, black-walled one. This was made certain by pouring the two lots simultaneously into white containers and making the comparison immediately, before color changes occurred. It is evident that in this experiment the bottom had exerted more influence than the walls, despite the fact that the former had an area of 491 square centimeters and the latter an area (below the water line) of 589 square centimeters. It must be noted, however, that the walls were far from being without influence. Fishes in the white-bottomed container were distinctly darker than those kept in an all-white one, while fishes in the black-bottomed container did not become as dark as those in an all-black one.

In another experiment, carried out earlier, the bottom areas were much more restricted, in proportion to the walls. Small battery jars, 18 cm. high by 13 cm. internal diameter, were used. The water was 10 cm. deep. Here the ratio of bottom surface to wall surface below the water line was about 1 to 3. Since running water was not used, the fishes probably swam near the surface much of the time. Five jars of each sort were employed, each containing two specimens. Careful comparisons by two observers were made on several different days, the fishes on each occasion being poured simultaneously into all-white jars. In every case the fishes from the black-bottomed jars were darker.[5]

The experiments hitherto described are open to one objection, namely, that the visual field is continually changing with the movements of the fish, or even with the movements of its eyes. Thus areas of very different degrees of illumination may be imaged in turn upon the same region of the retina. If different areas of the retina have different potencies, in relation to their control over the chromatophores, the experimenter must devise a method by which each region may be subjected more or less continuously to the same stimulus. It is the chief purpose of the present paper to discuss results obtained by the use of one of these methods.

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[4] The depth of water, controlled by siphon outlets, was here about 7.5 cm.

[5] It is only fair to mention that a later trial of this experiment, with only one jar of each type, gave a result which was not in accord with the preceding. Individual propensities to swim at different levels may account for these differences.

Von Frisch (1911) described the results of some experiments in which he had covered the eyes of trout partially or completely with a mixture of vaseline and lampblack. This substance, he tells us, commonly remained in place for a quarter of an hour or less, after which it fell off.[6] In brief, von Frisch's findings were as follows:

(1) Covering both eyes completely gave varying results, but in the majority of cases a decided, though not maximal, darkening occurred.

(2) Covering one eye completely resulted in the fish's becoming extremely dark; thus, darker than when both eyes are covered. In the majority of cases the darkening of the body was unsymmetrical, the side opposite to the covered eye being the darker.

(3) Covering the lower half of both eyes likewise led to a very dark condition.

(4) Covering the upper half of both eyes, on the contrary, had no effect upon the coloration. The fishes (which, during the experiment, were on a white background) retained their pale shade.

(5) Covering the lower half of one eye led to a pronounced unsymmetrical darkening; covering the upper half of one eye gave no result.

Von Frisch's method was inadequate in at least two respects. In the first place, the duration of treatment which it allowed was hardly sufficient for definitive results. In the second place, it was not possible, as he himself admits, to be sure that the covering was completely impervious to light. Any foreign object of this sort is soon detached by the secretion of mucus on the eye, and it probably separates partially from the surface sometime before it actually falls off.

Early in 1931, the present writer commenced an analogous but much more extensive series of experiments. After considerable trial and error, the following method was developed. Since it may be of value to other investigators, it seems worth while to describe this rather fully.

Gelatin medicinal capsules (chiefly the "No. 0" size, 7 to 8 mm. diameter) were dipped repeatedly into a solution of celloidin of medium thickness, the solvent being allowed to dry somewhat between times. After several layers of celloidin were accumulated in this way, the desired pattern was painted upon the rounded end of the capsule[7] in India ink by means of a fine brush. This also was repeated a number of times to give the necessary opacity. After allowing the ink to dry[8] the capsules were dipped several times more into the celloidin solution. It was highly important to lay on enough of this substance to make the final product sufficiently rigid. The celloidin was again thoroughly dried, after which the gelatin of the capsules was dissolved out in water at about 50° C. The celloidin coatings were now ready for trimming. A convex circular area was cut out from the end of each, in size somewhat larger than the exposed surface of the eye of the fish to be experimented upon.

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[6] Parker and Lanchner (1922) covered the eyes of *Fundulus heteroclitus* with opaque screens, produced by pouring over them a mixture of collodion and lampblack, held in position by threads which had previously been stitched around the margins. Such drastic treatment would, however, seem likely to interfere with the normal responses of the fish.

[7] Both the body of the capsule and the cover may be used for the purpose.

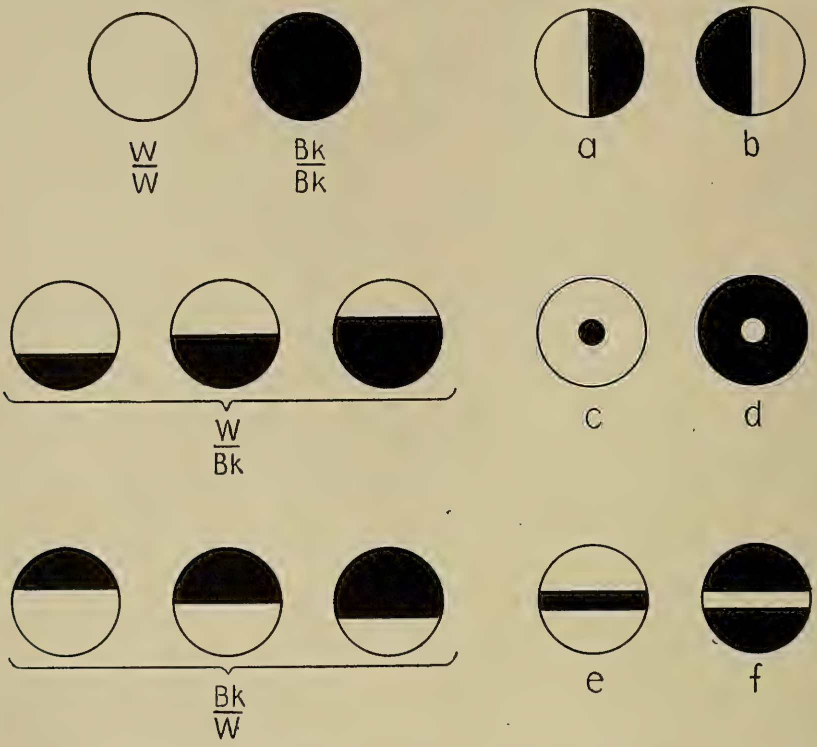

Fig. 1. $\frac{W}{W}$, $\frac{Bk}{Bk}$, $\frac{W}{Bk}$, and $\frac{Bk}{W}$, false corneas, wholly transparent, wholly opaque, and ones completely shading the lower and upper portions of the visual field (all uncolored). *a, b, c, d, e,* and *f,* additional types of "cornea" employed in a limited number of experiments. Dark regions completely opaque.

In practice, these celloidin eye-caps, which I shall speak of as

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[8] A drying oven at low temperatures was used for this and for drying the celloidin. The capsules were placed upon rows of pegs, consisting of finishing-nails driven into a board.

" corneas," were fitted into the front of the orbit of the fish, much as a glass eye is fitted into the orbit of a human subject. The fish was first anesthetized by being placed for a few minutes in a 1 per cent solution of urethane in sea water. No cutting of the tissues was necessary, though the conjunctiva was unavoidably lacerated somewhat by the edge of the celloidin cap. In most cases, these " corneas " remained in place for some hours at least, and frequently for a day or more.[9]

The accompanying diagrams (Fig. 1) show the types of uncolored " corneas " which I employed, i.e., ones upon which only black India ink (in the first case none at all) was used. Along with these diagrams are given the symbols for each which will be used in the ensuing text.

The $\frac{W}{W}$ was a cap of transparent, uncolored celloidin. The $\frac{Bk}{Bk}$ was intended to be completely opaque. In the later experiments, this point was tested by rather careful examination. The $\frac{W}{Bk}$ and $\frac{Bk}{W}$ were commonly divided into closely equal areas, so that when fitted into the orbit, the horizontal line of division passed approximately through the center of the pupil. Some were made, however, in which the opaque areas covered approximately one-third and two-thirds of the surface, respectively. When these were in position, the margin of the black area would just reach the pupil, in the one case, or just expose the edge of it in the other.

Certain other types of " cornea," which were used in only a few experiments, are represented on the right-hand side of the figure. The first two are obviously merely the $\frac{Bk}{W}$ or $\frac{W}{Bk}$ rotated through an angle of 90°. In *c* and *d*, the opaque spot and aperture, respectively, are of about the diameter of the pupil, while in *e* and *f*, the bar and the cleft are likewise intended to be of the width of the pupil. In practice, no such exact correspondence in dimensions was found possible.

Many experiments were also conducted with variously colored corneas. For this purpose Higgins's colored drawing inks were used. These, in thin layers, were found to be sufficiently transparent, though, needless to say, the character of the light thus transmitted was very imperfectly known. In the case of yellow, a much more satisfactory result was obtained later by using celloidin colored by picric acid. These colors were used singly or in almost every possible combination with one another or with white (i.e., uncolored areas) or black. Thus I had $\frac{Y}{Y}$ (all yellow), $\frac{Bk}{Y}$ (black above, yellow below), $\frac{W}{Y}$ (colorless above, yellow below), $\frac{Bl}{Y}$ (blue above, yellow below), etc.

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[9] In one case, they were removed after remaining seven days over both eyes. After a day or two, however, results cease to be very instructive, owing to injury to the eyes.

Experiments with these "goggles," as they were familiarly called, were commenced early in 1931, and have been conducted at intervals almost until the present time of writing. Altogether, several hundred of the "corneas" have been prepared, and records obtained from more than 200 fishes. As in the case of most biological experimentation which is carried beyond its initial stages, baffling contradictions and inconsistencies have been encountered, and for some of these no reconciliation has yet been found. This fact has been responsible for continual delay in my publication of these results, but present circumstances make it undesirable that I should postpone this report any longer.

The fishes here used were all of a single species, *Fundulus parvipinnis* Girard. Medium-sized or large specimens were used, ranging from 6 to 9 cm. in length. The sexes were not readily distinguishable during the winter and early spring, when most of these experiments were conducted. The effect of sex upon the color reactions at such times is very questionable. In the last of the series (May and June, 1933), females alone were employed, except for the purposes of comparison.

In a small proportion of cases, the fishes were tested before the experiments, in order to determine whether their chromatophore reactions were normal. This precaution was not generally taken, however, since otherwise healthy individuals seldom or never failed to respond in a striking manner to the shades of background here used. Much more frequently, a test of this sort was made after the fish had been subjected to experimentation. In some of these cases, the damage inflicted was found to have greatly impaired the animals' capacity for response.

I shall first consider those experiments into which the color element does not enter, i.e., ones in which the "corneas" have been coated with black ink or none at all. It may be useful to commence with comparisons between my own results and von Frisch's, in those cases in which we have performed analogous experiments.

In agreement with von Frisch, I found that when both eyes were covered with $\frac{W}{Bk}$ corneas—at least, when the upper and lower areas were approximately equal—the result was invariably a high degree of darkening on the part of the fish, regardless of the background on which it was placed. This, indeed, was the most constant result obtained in the course of these experiments. The fishes (14 cases) were invariably much darker than controls, and were in most instances of nearly maximum darkness. At the same time, the scale pattern was commonly pronounced, owing to the expansion of the chromatosomes (Sumner, 1933) belonging to the melanophores which outline the scales. This is in contrast with the effects of some stimuli, which lead to the effacement of the scale pattern, even though the fish may remain fairly dark.

In a limited number of cases, as already stated, the shaded and unshaded portions of the "cornea" were not of equal extent. In two out of three cases in which the shaded area just reached the pupil, a less pronounced darkening resulted than when the field was equally divided. In two out of three cases in which the pupil was nearly or quite covered,[10] a more pronounced darkening resulted.

I am also in agreement with von Frisch in finding that covering both eyes completely with opaque screens resulted in a decided, though not maximal, darkening of the fish. I have records of 11 cases. Like von Frisch, I found considerable variability in the outcome of these experiments, even in the later ones (6 cases) in which care was taken that the blinders were completely impervious to light.[11] Von Frisch conjectures that, even when the eye is completely covered, the retina may be stimulated by light entering through the translucent tissues of the head. For this I find strong evidence. In a number of cases, fishes with $\frac{Bk}{Bk}$ eye-caps, including some of known opacity, were found to undergo unmistakable changes of shade when transferred from white to black containers and vice-versa. The changes were in the same direction as those displayed by normal fishes, though much less in degree.

In some other respects, on the contrary, the results of my experiments upon *Fundulus* are in sharp disagreement with those of von Frisch upon the trout. Covering one eye completely led to a less pronounced effect than covering both eyes. This experiment was performed deliberately four times, and came about unintentionally in a number of other cases through the accidental detachment of one blinder. Throughout the entire course of these experiments, indeed, the loss of one of the "corneas" was a frequent occurrence. In general, it may be said that the covering of one eye by any type of cornea led to a result intermediate between that expected from the given type of experiment when both eyes were covered and that shown by the control fish upon the background in question. Frequently, indeed, the fish with one eye covered was almost indistinguishable from a control. Never was it recorded as displaying a more extreme condition than did the same fish when both eyes were covered.

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[10] I.e., hidden from view. In reality, considerable light must have entered from above.

[11] So far as revealed by a hand lens.

I have likewise failed to note any trace of asymmetry in the chromatophore reactions of fishes, in consequence of covering a single eye. This was watched for closely during the later stages of the experiments.

Von Frisch obtained no results of significance from experiments in which the upper half of each eye was covered. He reports only three such cases, and apparently in each instance the fish was returned to a white background and not tested upon any other. This last circumstance, I believe, was responsible for von Frisch's failure to observe a highly significant phenomenon.

The $\frac{Bk}{W}$ experiment was performed by me 34 times, including only those cases in which the cornea was divided about equally between the opaque and the transparent areas. Of these, more than 25 yielded significant results, the rest being inconclusive, owing to complications of an irrelevant nature.

So long as they were kept in white containers, these fishes, in most cases, resembled the controls rather closely, or they were sometimes slightly darker than these. When transferred to a dark neutral gray,[12] on the other hand, they commonly remained distinctly paler than the background, and paler accordingly than control specimens, which usually conform themselves rather closely to backgrounds of this shade (Fig. 2).

In cases in which the darkened area of the cornea just reached the pupil, a paling effect ensued which, however, was less marked, in two fishes out of three, than when the dark and clear areas were of equal extent. In three cases, on the contrary, in which the darkened area nearly or quite covered the pupil, the fishes were distinctly darker than when this was of smaller extent.

Since these results with $\frac{Bk}{W}$ corneas are perhaps the most significant ones reported in this communication, they deserve some further discussion. Taken at face value, they seem to furnish strong support for the hypothesis that the chromatophore reactions of fishes are determined by the relative luminosity of the lower and upper portions of the visual field, the former stimulating the chromatophores in a positive sense (i.e., in the direction of conformity), the latter in a negative sense. And despite certain complicating circumstances, I believe that this is, in reality, the most probable interpretation of the results.

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[12] This was closely similar to the "slate color" of Ridgway's "Color Standards and Nomenclature."

Unfortunately for the solution of this problem, however, the *lower field: upper field* ratio is only one of several factors which influence the shade assumed by these fishes during an experiment, and it is not easy to disentangle the part played by these various factors. If this ratio alone determined the result, one might expect that a fish, swimming over a well-lighted gray background, with total darkness everywhere above the horizon, should assume a maximum degree of pallor. In reality, the fish seldom does this. It is not unlikely, however, that this difficulty may be only an apparent one. The probability has already been pointed out that considerable light reaches the retina through va-

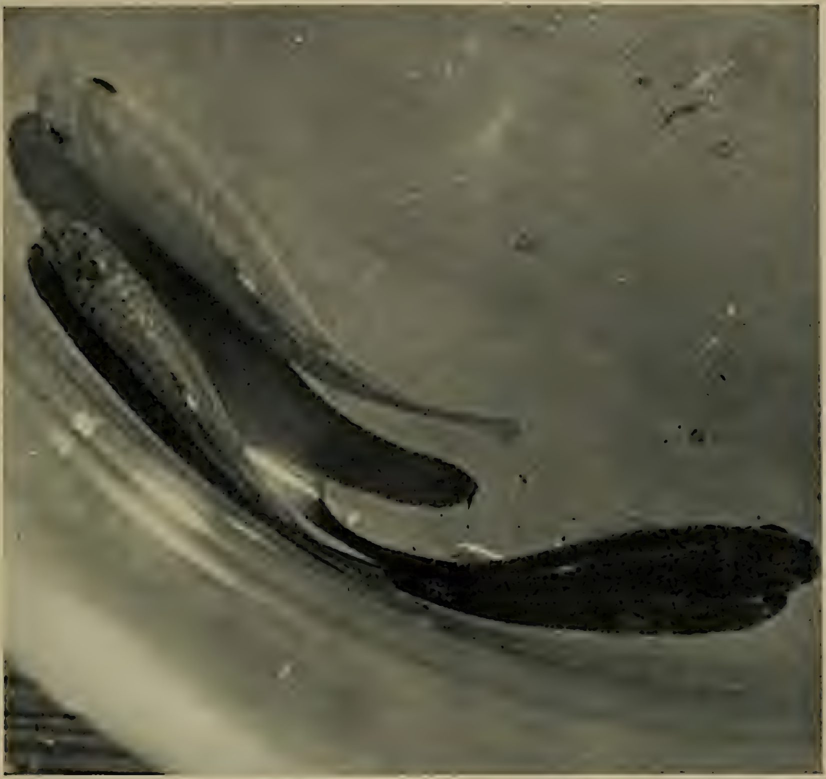

FIG. 2. Fishes wearing $\frac{W}{Bk}$ and $\frac{Bk}{W}$ corneas, along with a control. The first will be recognized as the darkest, the second as the palest, the control being intermediate. For photographic reasons, the fishes all appear somewhat too dark, relative to the background.

rious tissues of the head, beyond the margins of the false cornea. It is thus probable that the upper half of the visual field is not totally dark in these experiments.[13]

Again, it is known that the absolute degree of illumination has a decided influence upon the shade assumed by many fishes, irrespective of the background. This can be observed, in the case of *Fundulus,* by watching them as they swim in and out of shaded places. The $\frac{Bk}{W}$ specimens in the foregoing experiments exhibited this phenomenon in a high degree. They frequently assumed a grayish appearance while on the shaded side of the jar, becoming intensely pale upon emerging into the light.

A more serious complication, in our endeavor to interpret these experiments, arises from the unavoidable presence of stimuli due to the mechanical pressure of the eye-caps themselves, as well as to the suppression of all movements of the eyes. To test the effects of these stimuli, a considerable number of fishes (15) were fitted with " corneas " of clear, uncolored celloidin. The majority of these specimens became recognizably different in appearance from controls, the difference commonly lying in the direction of increased pallor, when on gray. In one of the cases, indeed, it is recorded that such a fish was even paler than the $\frac{Bk}{W}$ specimens which were available at the time for comparison.[14]

This circumstance doubtless weakens our argument based upon the effect of the $\frac{Bk}{W}$ corneas. Mechanical pressure upon the eyes tends to cause contraction of the melanosomes of the body. Some stimuli (e.g., darkening of the lower part of the visual field) are sufficient to overcome this effect. Where there are no opposing stimuli, it persists. May not this be the entire explanation?

That this explanation is not a satisfactory one is indicated by the fact that these cases of extreme paling were seldom met with among fishes with transparent colorless corneas ($\frac{W}{W}$), while they occurred not only in a large proportion of the $\frac{Bk}{W}$ fishes, but in some of those in which the upper halves of the corneas were merely colored deeply without being rendered opaque. In eight out of nine experiments in which direct comparisons are possible with $\frac{Bk}{W}$ and $\frac{Bl}{W}$ specimens, including those most recently and carefully performed, the latter are recorded as being distinctly paler than the accompanying $\frac{W}{W}$ ones.

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[13] Unpublished experiments upon *Lebistes* have shown that both eyes of a young specimen may be extensively damaged by electrocautery without seriously interfering with the capacity of the fish to adjust itself to black and white backgrounds. In many cases, the eyes were so badly damaged that a person thus affected would doubtless rate as " totally blind." When complete destruction of the eye was accomplished, on the other hand, the capacity for response to background was entirely abolished.

[14] Leaving out of consideration another case, in which the available $\frac{Bk}{W}$ fishes had corneas 1/3 and 2/3 black, respectively, and remained relatively dark (see below).

While von Frisch detected no effect from darkening the upper half of the field of vision, he does not contend that this part of the field plays no rôle whatever in determining the chromatic reactions of the fish to optic stimuli. From the fact that a more extreme darkening is produced by covering the lower half of each eye than by covering both eyes completely, he concludes that the former response involves a contrast effect, the contrasted elements being, of course, the upper and lower halves of the field of vision. Similarly, the results which he reports from covering one eye of a fish, namely, a more extreme darkening than when both eyes were covered, were held to substantiate the same view. The receptor elements here concerned, if I understand him correctly, were the *lower* half of the covered eye (the upper half being irrelevant), and the *upper* half of the uncovered eye (the lower half being irrelevant).

Bauer (1905) had earlier adopted a similar contrast theory in interpreting the results of his experiments with isopod crustacea. If one half of the visual field was darkened while the other remained illuminated, the animal became intensely dark, and it mattered not, in these experiments, which half was darkened and which was illuminated. Von Frisch believes that the fish differs from the isopod, in that the visual field of the former is, in a sense, polarized, the upper and lower halves playing different rôles.

As stated above, my experiments with *Fundulus* furnish conclusive evidence, for this fish at least, that an opaque covering over one eye results in a darkening which is not only far from maximal, but considerably less pronounced than that which results from covering both eyes.

Furthermore, results from the limited number of experiments in which the light and dark areas were arranged as shown in Fig. 1, *a* and *b*, were not in harmony with the contrast theory, at least in the form in which it is stated by von Frisch. When the field of vision was thus divided by a vertical line into anterior and posterior halves, the result was in each case a slight or moderate degree of darkening. This was irrespective of whether the darkened half was anterior or posterior. The fish upon white was in some cases nearly as pale as the control, never was it of maximum darkness.

It is desirable at this point to include the results from the use of the four other types of cornea shown in Fig. 1, although their meaning is not clear. Two trials were made with each of these. Arranged in order of increasing darkness, the fishes, in both trials, lined up as follows on gray backgrounds: *e, c, f, d*. The first of these (*e*), in each case, was of a pallor approaching that of the $\frac{Bk}{W}$ fishes; the last (*d*) was of more than medium darkness. The data are insufficient for an interpretation of these results. As indicating that the lower half of the visual field exerts an influence in a positive direction upon the shade assumed by the fish, these results may be regarded as being in harmony with ones previously described. It will be noted that *d* and *f* were darker than *c* and *e*. On the contrary, the parts played here by the central and upper portions of the field are far from clear. Before reaching any safe conclusions, more numerous experiments would be necessary, in which the exact position of the pupils in relation to the clear areas was carefully controlled.

While I believe that von Frisch is correct in his contention that the upper and lower halves of the visual field play different rôles in relation to the pigmentary responses of fishes, I believe that he is wrong in restricting the rôle of the upper half to that of intensifying by contrast, when bright, the effect of a darkened lower half. My evidence as a whole seems to show that a darkened upper half has a definite negative effect in causing the fish to react to a background of medium or darker shade as if this were very much paler than it is in reality. This effect is complicated, it is true, by the influence of other stimuli, so that it is not always clearly recognizable.

If the upper and lower portions of the retina really have different potencies in determining the color reactions of fishes, this fact might be expected to reveal itself if, in any way, these areas could be transposed and the visual field thus inverted. As a matter of fact, I virtually accomplished this inversion in the case of several specimens. The fish was anesthetized and each eye severed from its connections with the orbit, save for the optic nerve. The ball of the eye was then rotated on its major axis through an angle of 180°, and fastened in its new position by stitches or otherwise. But the operation proved to be too drastic, and none of the fishes were thereafter capable of making any chromatic responses whatever, although four specimens lived for some hours. Possibly a more skillful ophthalmic surgeon than I might have had better success.

An apparatus was also contrived, by which light could be projected into the eyes of fishes, held in position in special glass tubes made for the purpose. Small glass windows applied closely to the fronts of the eyes at the ends of brass tubes were so painted as to control the portions of the retina which were darkened or illuminated. Light was supplied by two electric flash-light lamps. Thus far, the results have not been very instructive, since the desired experimental conditions do not seem to have been fully realized.

In conclusion, I shall make rather brief mention of the experiments with colored corneas. No extended discussion of these experiments seems warranted, despite the very large amount of time which was devoted to them. This is owing to the inconclusive nature of many of the results. In my earlier experiments (January, February, and March, 1931) I seemed to find unquestionable evidence for a specific effect of yellow " goggles " upon the color of the fishes. In a considerable number of cases, a rather rich yellow resulted from this treatment, much as it did from keeping a fish for some hours or days upon a yellow background. Examples of such an effect were shown at various times to members of the Scripps Institution staff, as well as to visitors, and records of these experiments make it evident that this result was regarded at that time as the rule.

This marked yellowing of the fishes, due doubtless to the contraction of the melanosomes and simultaneous expansion of the xanthosomes, followed the insertion of $\frac{Y}{Y}$ corneas, as well as of $\frac{Bk}{Y}$, $\frac{W}{Y}$, and $\frac{Bl}{Y}$ ones. The effect would thus seem to be the result of stimulating the whole or a part of the retina by light of certain wave lengths. No contrast between different parts of the visual field was required. This is, of course, in harmony with the findings of von Frisch and others in experiments with the effects of monochromatic light.

Red likewise appeared to bring about a characteristic chomatic response, the fish becoming not intense red, but pale reddish brown, of the same shade as is assumed by this species when kept in a red container. Green and blue brought about no probable specific color changes, though blue corneas induced a neutral or sometimes slightly bluish gray, which differed distinctly from the buff or yellow appearance of fishes exposed to yellow.

In harmony with the previously described experiments, in which the upper or lower portions of the corneas were rendered entirely opaque by black ink, those which were colored above or below exerted, in many cases, an influence upon shade, whether or not a specific color effect was shown. Thus, $\frac{Bl}{W}$ (blue over white) sometimes led to a condition paler than controls; $\frac{W}{Bl}$ to a condition darker than these.

In view of the apparently convincing nature of some of the foregoing experiments with yellow or partially yellow corneas, it was decidedly disconcerting, in a recent rather prolonged series of experiments, to encounter an almost complete absence of such effects. In order to determine whether these negative results were due to a change in the reactions of the fishes to color stimuli from any source, I next subjected a considerable number of fishes from the same stock, and of both sexes and various sizes, to the influence of yellow aquaria. Little or no specific color effect was now manifest. This was surprizing, in view of the striking responses to yellow backgrounds which had been manifested some months earlier,[15] and which were made the basis of experiments upon the possible effects of visual stimuli on xanthophyll formation (Sumner and Fox, 1933).

It is probably significant, in relation to these contradictory findings, that the first three of my series of experiments were conducted during the months of January, February, and March, while the last was carried on in late May and early June.[16] Some unknown metabolic change, due to season, must be credited with this undoubted difference in the chromatic reactions of these fishes.

Before closing, it should be stated that we unduly simplify the reactions of these fishes when we speak merely of their turning " pale " or " dark." The melanophores at different depths of the tissues respond more or less independently of one another. A fish may show little melanin in the superficial melanophores and yet remain fairly dark. When this deeper pigment disappears from view a translucent condition ensues. Likewise, the xanthopores react independently of the melanophores, and differences of color as well as of shade appear which frequently have nothing to do with the color of the background. The same fish, in its deeper tissues, may appear yellowish, greenish, or bluish, or may have a curious coppery tint, all without known causes. These changes naturally complicate any interpretations of experimental results. Anesthetization of the fish with urethane results not only in a marked darkening, due to the expansion of the melanosomes, but commonly leads to a conspicuous display of the yellow pigment as well. These effects frequently do not disappear for a half hour, or even considerably more, after apparent recovery of the animal from the anesthetic, a fact which must be borne in mind by the observer.

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[15] Various students of chromatophores, e.g., Fries (1931) have likewise noted the marked effects of yellow backgrounds upon *Fundulus heteroclitus.*

[16] I have records showing that *Fundulus* gave pronounced responses to yellow-walled containers during every month from October to February, inclusive (October 23–March 1).

SUMMARY

(1) Darkening the lower half of the field of vision by the insertion of false corneas of celloidin resulted in a pronounced darkening of the entire dorsal surface of the fish (*Fundulus parvipinnis*), even when the animal was kept upon a white background.

(2) Darkening of the upper half resulted in no visible change (unless a slight darkening) when the fish was on white, but a fish so treated was commonly considerably paler than a control when placed upon a dark gray background. This fact, along with that stated in the preceding paragraph, seems to support the view that the shade which a fish assumes upon a given background is determined by the relative luminosity of the upper and lower portions of the visual field, the latter acting in a positive sense, the former in a negative.

(3) Various other stimuli, optical and otherwise, were found to affect the shade assumed by the fish, and these greatly complicated the interpretation of the experiments.

(4) Covering both eyes with opaque "blinders" resulted in the assumption of an intermediate shade. Contrary to the findings of von Frisch with the trout, the covering of one eye, in a fish kept on a white background, resulted in only a slight darkening. Furthermore, no trace of asymmetrical chromatophore response on the body was noted, following asymmetrical optical stimulation.

(5) In experiments during the winter and early spring, it was found that transparent "corneas" which were stained yellow, either throughout their entire extent, or in the lower half only, led to the assumption of a distinctly yellowish hue by the fish. In another series, some months later in the year, little evidence of such color changes was found. This accorded with the fact that these fishes responded markedly to yellow backgrounds during the earlier months, and ceased to do so during the later ones. Some physiological change of a seasonal nature appears to be involved here.

(6) Besides yellow, and to a lesser degree red, no other color evoked any specific color responses on the part of the fish. On the contrary, various color changes were manifest which obviously bore no relation to the chromatic stimuli.

PAPERS CITED

Bauer, Viktor, 1905. Ueber einen objektiven Nachweis des Simultankontrastes bei Tieren. *Zentralbl. für Physiol.,* **19**: 453.

Fries, E. F. B., 1931. Color Changes in Fundulus, with Special Consideration of the Xanthophores. *Jour. Exper. Zool.,* **60**: 389.

Frisch, Karl von, 1911. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. *Pflüger's Arch. für die ges. Physiol.,* **138**: 319.

Keeble, F., and F. W. Gamble, 1904. The Colour-physiology of Higher Crustacea. *Phil. Trans. Roy. Soc., B,* **196**: 295.

Mast, S. O., 1916. Changes in Shade, Color, and Pattern in Fishes, and their Bearing on the Problems of Adaptation and Behavior, with especial reference to the Flounders Paralichthys and Ancylopsetta. *Bull. U. S. Bur. of Fish.,* **34**: 173.

Parker, G. H., and A. J. Lanchner, 1922. The Responses of Fundulus to White, Black and Darkness. *Am. Jour. Physiol.,* **61**: 548.

Pearson, J. F. W., 1930. Changes in Pigmentation Exhibited by the Fresh-water Catfish, Ameiurus melas, in Response to Differences in Illumination. *Ecology,* **11**: 703.

Sumner, F. B., 1911. The Adjustment of Flatfishes to Various Backgrounds: a Study of Adaptive Color Change. *Jour. Exper. Zool.,* **10**: 409.

Sumner, F. B., 1933. Why Do We Persist in Talking About the "Expansion" and "Contraction" of Chromatophores? *Science.* In press.

Sumner, F. B., and D. L. Fox, 1933. A Study of Variations in the Amount of Yellow Pigment (Xanthophyll) in Certain Fishes, and the Possible Effects upon this of Colored Backgrounds. *Jour. Exper. Zool.* (In press.)

Sumner, F. B., and A. B. Keys, 1929. The Effects of Differences in the Apparent Source of Illumination upon the Shade Assumed by a Flatfish on a Given Background. *Physiol. Zool.,* **2**: 495.

SEXUAL PHASES IN TEREDO

WESLEY R. COE

Osborn Zoölogical Laboratory, Yale University

The great disparity in the sex ratios reported for various species of shipworms indicates that the sex-differentiating mechanism must function somewhat differently than it does in animals that exhibit an equality or near equality of the two sexes. If examined during the breeding season the adults appear to be of separate sexes and frequently the females are many times more numerous than the males. More than eighty years ago Quatrefages reported a ratio of only five or six males in each hundred individuals of *Teredo norvegica*. Other investigators have claimed that various species of the genus are hermaphroditic. In Barnegat Bay, New Jersey, Nelson (1922) estimated that there was only one male to about every five hundred females of *Teredo navalis*. Similar statements appear in many treatises and textbooks. Grave (1928) found a much smaller excess of females at Woods Hole, Massachusetts, but May (1929) discovered only a single male among the individuals of *T. navalis* which he examined from the coast of Germany.

It has often been observed, however, that the mean size of the females considerably exceeds that of the males and it has been suggested that this disparity in size may be due to sex reversal of protandric females. On the other hand, the observed size differences might be assumed to result from a different metabolic rate in the two sexes, or perhaps the sex is controlled by the physiological conditions at the critical time of sexual differentiation. Only a few direct observations have been made to determine which, if any, of these alternative possibilities may be responsible. Yonge (1926) reports the examination of between two and three hundred adults of *T. norvegica* in summer at Plymouth, England, but found only separate sexes. At the same locality in February he found two hermaphrodites among many individuals of separate sexes and concluded that these two were in the act of changing from male to female. This was considered adequate evidence of protandry. Sigerfoos (1908) found many hermaphrodites among young individuals of *Bankia gouldi* and suspected that protandry is normal in that species of shipworm. Kofoid and Miller (1927) observed that males of *T. navalis* (*T. beachi* Bartsch) in San Francisco

Bay are commonly smaller than females, but found no evidence that an actual change of sexual phase might occur.

It is obvious that the question can be settled only by following the growth stages during the entire life and under a variety of environmental conditions. Such is the object of the present investigation.

In a recent publication (Coe, 1933), reporting the destruction of mooring ropes in Long Island Sound by *Teredo navalis morsei* (*T. morsei* Bartsch, 1922), mention was made of the evidence for protandry in that species. Rope is such an unnatural and unsuitable material for the sustenance of shipworms that normal growth is inhibited and only dwarf, or stenomorphic animals are found. Yet such individuals may reach sexual maturity and produce a relatively small number of normal gametes. All young individuals appear to pass through a functional male phase and most of them later become females.

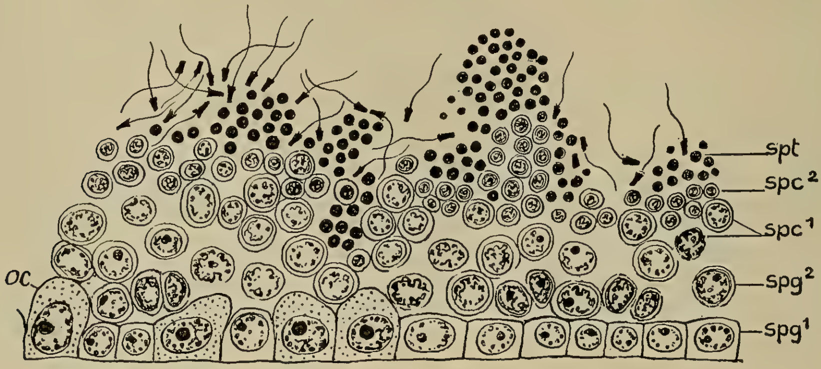

FIG. 1. Gonad of initial male phase, male type, with a few spermatozoa already formed; cortical layer with only a few small differentiated ovocytes (*oc*); *spg*¹, *spg*², primary and secondary spermatogonia; *spc*¹, *spc*², primary and secondary spermatocytes; *spt*, spermatids.

Histological study shows that the developing gonad is always more or less distinctly bisexual, each follicle consisting of an outer or cortical layer of ovocytes with a mass of spermatogenic cells filling the lumen (Figs. 1–3). Proliferation of spermatogonia proceeds rapidly and spermatogenesis follows so quickly that the gonad may be filled with ripe spermatozoa within five to six weeks after the young animal has completed metamorphosis. Before the spermatozoa have been discharged, however, the ovocytes in the cortical layer have begun the deposit of yolk in anticipation of the following female phase (Fig. 3). Entirely parallel conditions are found in *T. navalis novangliæ* at Woods Hole (Figs. 4–6).

In both these varieties there is much variation in the relative proportion of cortical ovocytes and medullary spermatic tissue, with a fairly definite correlation between the early deposition of yolk and the abundance of male cells. With the exception of older animals which had

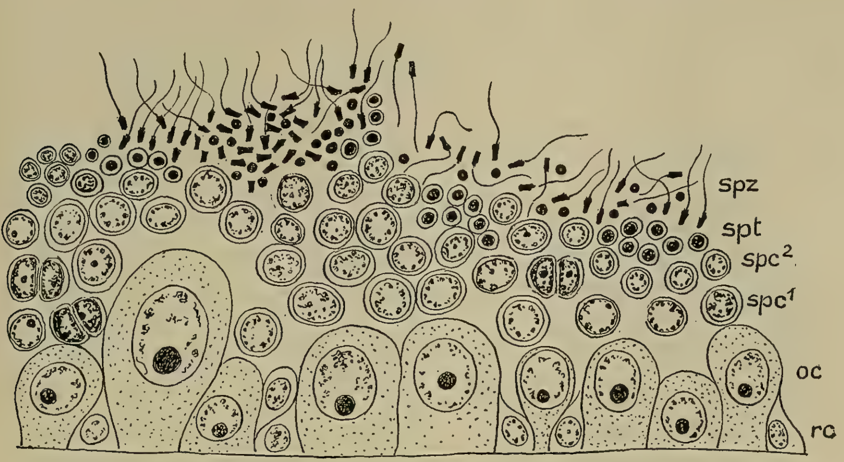

FIG. 2. Primary gonad of protandric female, with cortical layer of yolk-forming ovocytes and numerous ripe spermatozoa (*spz*) in the lumen of the follicle; *rc*, residual cells (mainly smaller ovocytes) which form the germinal cells of the gonad after the first ovulation; other letters as in Fig. 1.

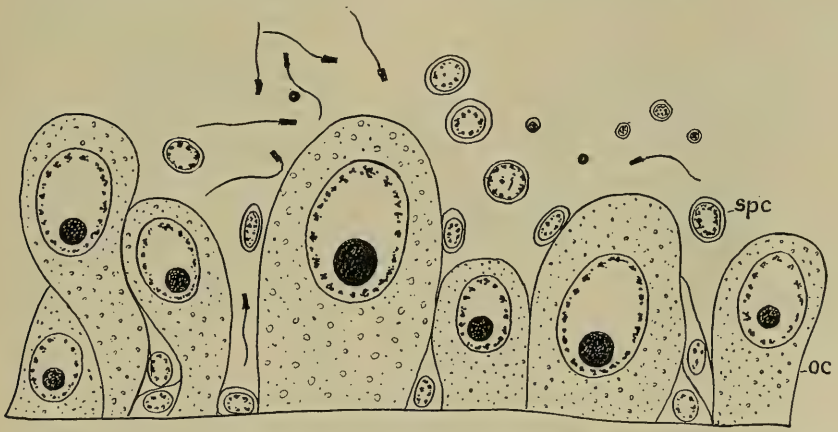

FIG. 3. Later stage in the gonad of protandric female preceding the first spawning period, showing the cortical layer of large ovocytes (*oc*) and small residual cells, with a few spermatocytes (*spc*), spermatids, and spermatozoa from the initial male phase remaining in the lumen.

previously completed the male phase and showed only female characteristics, both types of sexual cells were present in nearly every one of more than two hundred gonads cut in serial sections. The youngest sexually mature individuals would naturally be classed as males and the oldest as females, with an intergrading series between.

The great variation in the proportion of cells characteristic of the two sexes that is also found in animals of apparently the same age indicates either an environmental influence or a dominating action of the genetic factors of one sex or the other. In general, the growth of the cortical ovocytes seems to inhibit to some extent the proliferation of the spermatogenic cells, possibly through the assimilation of all the available nutriment. Thus the gonads of some individuals at the time when the first spermatozoa are formed may have a characteristically male appearance, with only a few scattered ovocytes of small size (Plate I, Fig. 8) or they may more closely resemble ovaries, each follicle having

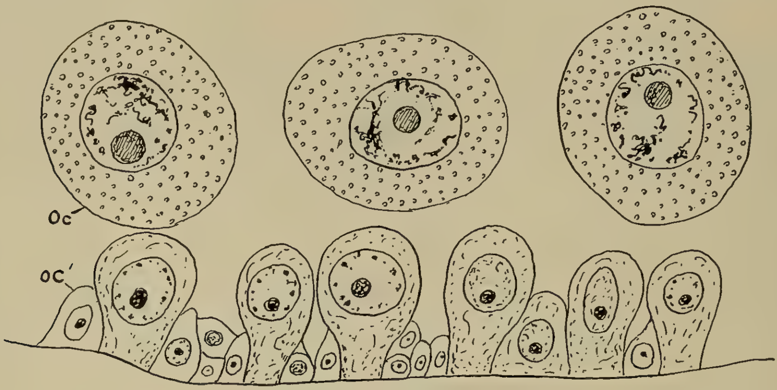

FIG. 4. Ovary at time of first ovulation, showing half-grown residual ovocytes (*oc*^{1}) and small indifferent cells remaining after the discharge of the ripe ova (*oc*).

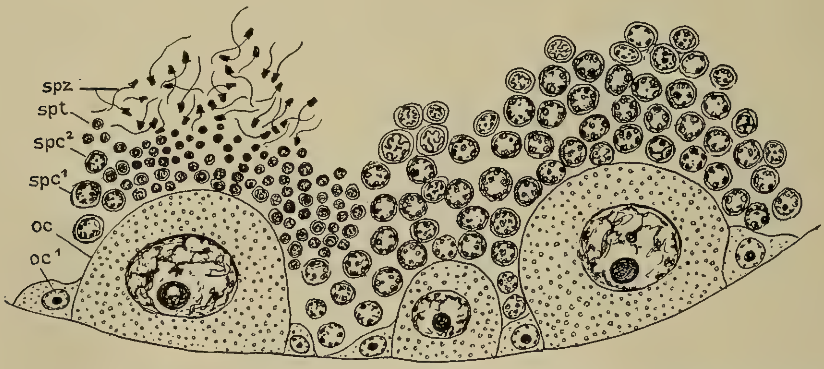

FIG. 5. Initial male phase of gonad of protandric female with large ovocytes (*oc*) which reach maturity as soon as spermatogenesis has been completed. Smaller ovocytes (*oc*^{1}) form the ova of the second ovulation. Other letters as in Fig. 1.

a complete cortical layer of large ovocytes enclosing relatively few spermatogenic cells in the lumen (Plate I, Fig. 7; Plate II, Fig. 10). Not infrequently a more definitely hermaphroditic condition exists (Plate II, Figs. 11, 12) or various parts of the same gonad may differ in these respects (Fig. 6).

In addition to the protandric females which under favorable conditions constitute the vast majority of the population, there occur a few individuals which appear to retain the male phase through life.

These may be called true males to distinguish them from the male phase of protandric females. Most of these true males remain much smaller than the females of the same age but some of them may be as large as the average female. Selection for size alone would thus segregate most, but not all, of the males in a group of uniform age. The proportion of such males in a population seems to be greatest where the conditions for rapid growth are least favorable, indicating, perhaps, that the environment may influence the expression of the sex-differentiating mechanism in the teredo as it appears to do in the oysters (Coe, 1932*a*, and *b*).

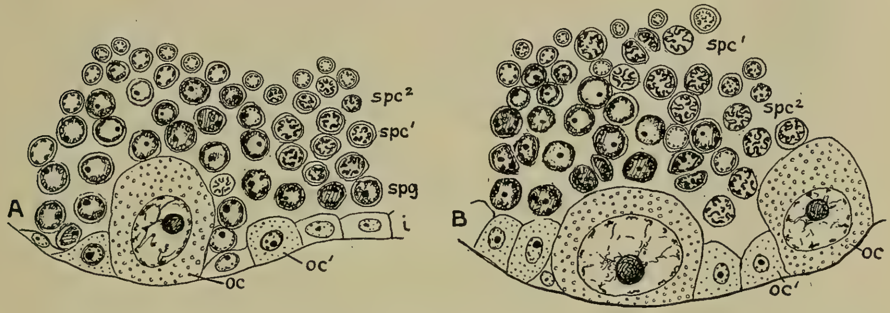

FIG. 6. Portions of two follicles from the same gonad, showing variation in the relative proportions of ovocytes (oc, oc^1) and spermatogenic cells; *i*, indifferent residual cells. Other letters as in Fig. 1.

It has been repeatedly observed that after the animal reaches the female phase the first ovulation may take place while the body is relatively small. Only a few hundred ova are then produced, although the later crops may each include more than a hundred thousand.

Several crops of ova may ripen in each well-nourished animal during the breeding season which extends over a period of about five months. As the size of the body increases the number of ova at each ovulation tends to become greater.

In this species the ripe ova pass from the genital duct to the mantle cavity, where they are fertilized by spermatozoa brought into the body with the water which passes through the gills. During early cleavage the eggs reach the gill chambers and in this situation the embryos develop into larvæ which are later liberated into the water as free-swimming veligers (Nelson, 1922; Kofoid and Miller, 1927; Grave, 1928). Several thousands of these bivalve larvæ may be discharged from the body within the space of a few minutes.

The eggs usually, but not always, leave the ovary before the disappearance of the germinal vesicles. Contact with the sea water in the mantle cavity results in the rapid dissolution of the nuclear membrane and formation of the first polar spindle, if this has not already been formed in the ovary. The entrance of the spermatozoön leads to the formation of both polocytes. In contact with the gills the eggs complete cleavage, after which the young embryos become closely packed between the gill plates, where they continue development for two to three weeks.

After ovulation the follicles of the ovary collapse to some extent but large numbers of residual ova remain to form the basis of the second crop of ova (Fig. 4). A second ovulation commonly follows shortly after the first crop of veligers has left the branchial chambers or about three weeks after the first. Occasionally, however, particularly in the early part of the breeding season, the second crop of ova reaches the gills while many of the larvæ of the first crop are still present. It is estimated that the larvæ spend from two to three weeks in the free-swimming stage before settling upon the wood in which they metamorphose to the adult form. While the successive crops of young are being produced during the summer the animal has been rapidly growing in size and elongating its burrow in the wood, provided the nutritive conditions remain favorable and the extent of the remaining wood permits.

The life span of the teredo is seldom more than one year, but the rate of growth is very rapid under favorable conditions. Sexual maturity may be reached within six weeks after metamorphosis in the warmer part of the year, when the body has reached a length of 15–30 mm. Growth continues, however, and an animal a year old may have

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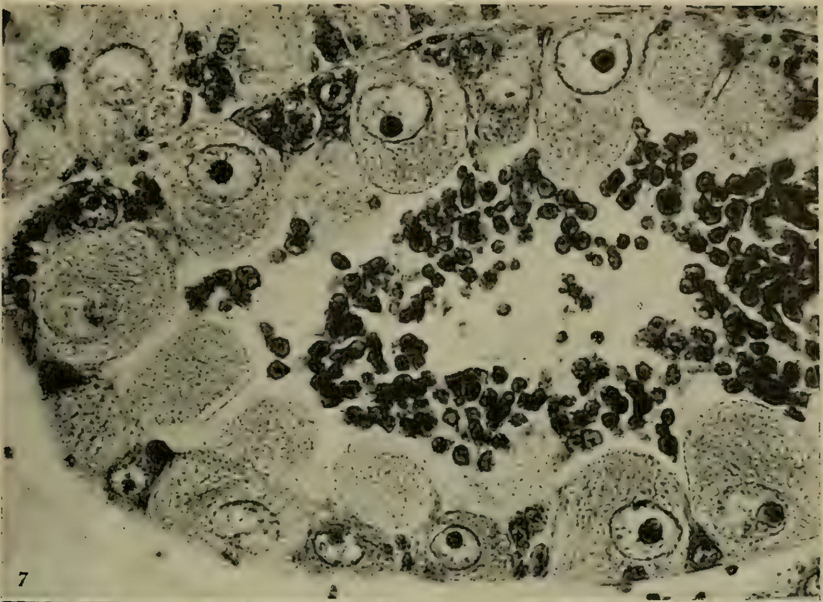
EXPLANATION OF PLATE I

FIG. 7. Primary gonad with strong female tendency, showing the cortical layer of large ovocytes, with a relatively small number of spermatogenic cells in the lumen.

FIG. 8. Primary gonad with strong male tendency, with only a few differentiated small ovocytes in cortical layer and abundant spermatogenic cells in the lumen.

FIG. 9. Initial male phase nearly completed; lumen of follicle filled with vast numbers of spermatozoa; cortical layer with half-grown ovocytes. Transition stage from male to female phase.

Plate I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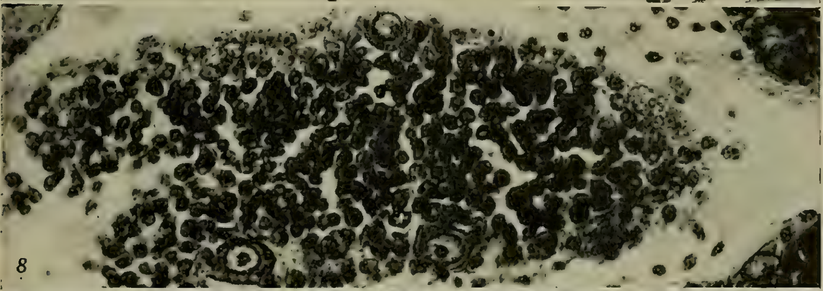
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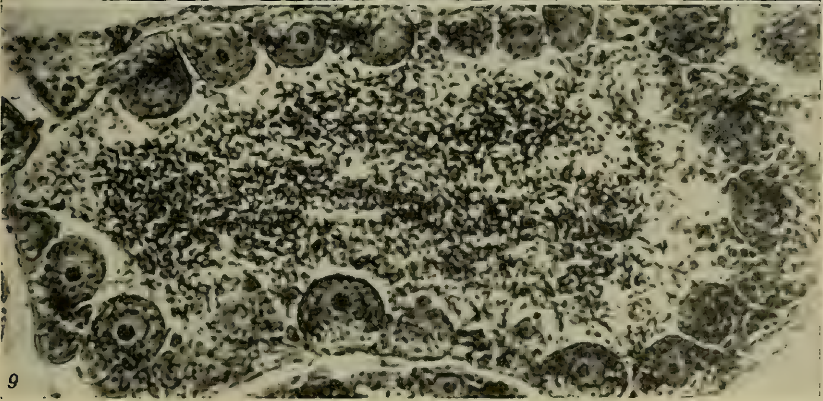
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attained the great size of 40 cm. in length and more than 9 mm. in diameter (Grave, 1928). Usually, however, the limits of the available wood do not permit so extensive a growth. All observers agree that relatively few sexually mature individuals survive their first winter and that death normally occurs before the end of the second winter in these localities, except in the case of those that were very immature during their first winter.

Several broods of young may have been produced in the meantime but there is no evidence of a second change of sex. Having reached the definitive female phase the individual evidently continues to function as that sex throughout the rest of its life.

The histological basis for the changes which the gonads undergo during the development of each of the sexual phases has been studied at all seasons of the year and under various environmental conditions. The principal features are reported on the following pages.

HISTOLOGICAL BASIS OF THE SEXUAL PHASES

Both at Woods Hole and in Long Island Sound the breeding season of the respective varieties of *Teredo* extends over a period of several months. The first ovulation may begin early in May for some individuals and the last usually continues into October for others. In a large and well-nourished individual the aggregate of the young produced may exceed a million. Larvæ are present in the water at all times during the breeding season (Grave, 1928).

Consequently, as sexual maturity may occur in the young animals within six weeks after metamorphosis, there are innumerable overlapping broods in the population of a locality each season. The conditions are further complicated by the fact that the metamorphosing young are likely to settle upon the same timbers that are already occupied by earlier arrivals. Only by placing pieces of wood in the water at frequent intervals can the ages of the occupants be ascertained.

If such a wooden trap be set in the spring in an infested locality and examined the following spring, animals of many different ages

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EXPLANATION OF PLATE II

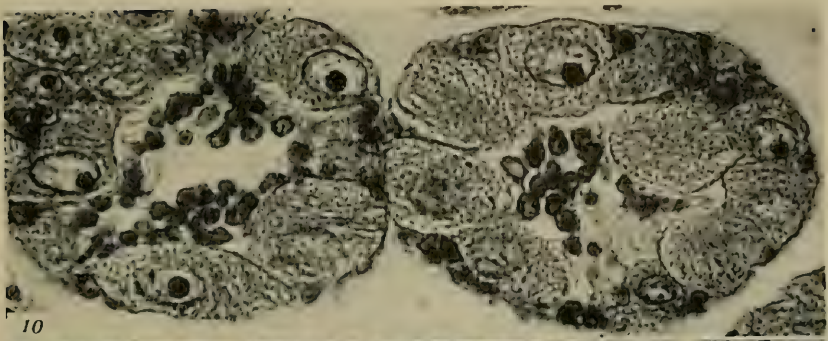
Sections of gonads of three individuals of *T. n. novangliæ* from Woods Hole, showing different aspects of sexuality in protandric females in early spring (March 29) and shortly before the onset of active spermatogenesis.

FIG. 10. Portion of gonad of feminine type of protandric female, with relatively few spermatogonia in lumens of follicles and large ovocytes on cortex.

FIG. 11. Portion of gonad of masculine type of protandric female, with numerous spermatogonia and spermatocytes in the lumens of the follicles. Ovocytes form complete cortical layer.

FIG. 12. Portion of gonad of the hermaphroditic type of protandric female, with only a few large ovocytes and numerous spermatogonia.

Plate II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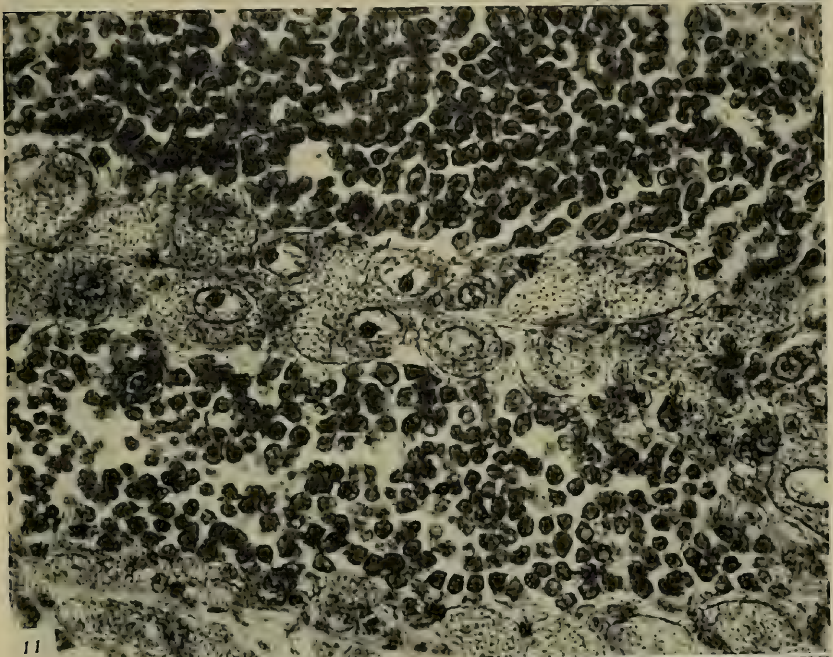
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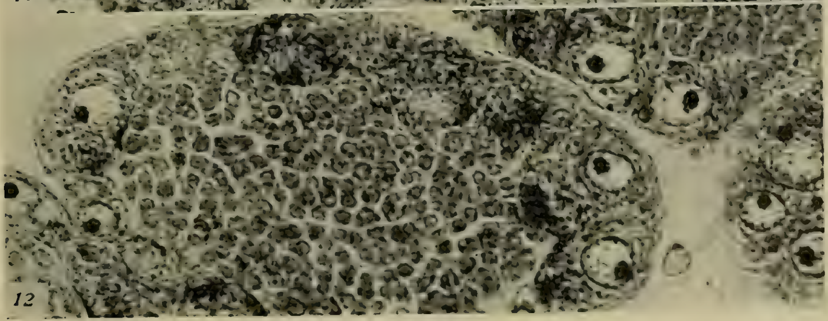
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and of different aspects of sexuality will probably be obtained. The large ones will show a preponderance of females while the smallest, if sexually differentiated, will be exclusively in the male phase unless the block is so greatly overcrowded that the differentiated females have had no space for growth. In such a locality, if unprotected piling or other wooden structures have been in the water for a year or more, eight or nine somewhat different phases of the life cycle of the teredo, all of which are sometimes found in a single piece of timber, may be present at the beginning of the breeding season: (1) Large adult females, nearly one year old, which completed the protandric male phase early the preceding summer. These are not usually very numerous but each may contain an enormous number of ova. (2) True males, of the same age as the oldest females; very few but filled with large masses of spermatozoa. (3) Younger females which completed the male phase during the middle or later part of the preceding summer, also with very numerous ova. (4) Small females which reached the female phase at the end of the last breeding season. (5) True males which shed one crop of spermatozoa at the end of the last breeding season. (6) Young hermaphroditic males, filled with spermatozoa but also containing half-grown ova, showing that these individuals represent the initial male phase of the protandric females; these are usually far more numerous than all the other types combined. (7) Young females in which the initial male phase may have been abortive. (8) Young true males, differentiated from the hermaphroditic males by the presence of only a few small ovocytes on the walls of follicles containing an unusually large number of spermatogenic cells. (9) Very small individuals which completed metamorphosis late in the autumn and have not reached sexual maturity; these may remain so small as to escape notice during the winter but their presence becomes evident in the spring. Not all of the phases enumerated can be easily distinguished from each other, for there are many intergrading stages associated with each phase (Plates I and II, Figs. 7–12) and the rate of growth is highly variable.

If the timber has been in the water only since the middle of the preceding summer the oldest groups will not be found, and if it was placed in the water toward the end of summer only the younger groups will be present.

Perhaps it may be assumed that in the teredo, as in other pelecypods, the necessary stimulus for ovulation is provided by the presence of spermatozoa of the same species after the water has reached the requisite temperature and that the spermatozoa are discharged by the sexually mature males through the stimulus of the rise of temperature alone. If such be the case there must occur a widely spread emission of sperm by the true males and especially by the more numerous protandric males at the beginning of the breeding season as soon as the temperature of the water becomes sufficiently high. This occurs during the first half of May in the localities here considered. Grave (1928) records this critical temperature as 11°–12° at Woods Hole, although Nelson (1928) finds that the teredo in Barnegat Bay, New Jersey, requires a temperature of about 15° to call forth the spawning reaction. The latter has generally been considered the critical temperature for the so-called *T. navalis* over the greater part of its supposedly extended range both in the Atlantic and the Pacific.

The individuals which reached the female phase during the preceding summer and autumn will respond one after another as their ova become fully mature. Two to three weeks later the first larvæ are liberated into the water and after a free-swimming period of about the same length of time a new infestation of the wooden structures in the vicinity begins.

As soon as the sperm of the much more numerous young protandric individuals are shed, their ova ripen and more abundant crops of larvæ are set free in the water day by day as the breeding season advances. A rapid rise in the temperature early in the breeding season may thus be followed by swarms of larvæ. A second period of excessive abundance is likely to occur shortly after these larvæ have reached the female phase of sexual maturity eight to ten weeks later. In the meantime the older females continue the supply of newly hatched larvæ daily. It has been noticed frequently that the teredo will mature sexually and produce a small number of gametes even if the wood is so overcrowded as to prevent the normal growth of the individual. Even under so unfavorable an environment as rope, when the entire population consists of dwarfs, sexual differentiation seems to follow its normal course and the protandric females produce a small number of ova after completing the functional male phase (Coe, 1933).

Although the expression of femaleness usually increases with advancing age, this rule appears to have some exceptions, for an occasional animal is found in which the hermaphroditic male phase is accompanied by the degeneration of ovocytes previously formed. It seems not improbable that such degeneration may occasionally include all the ovocytes and if such is the case a true male phase might follow a bisexual phase in which many ovocytes had already begun the deposit of yolk. It is obvious that the gonad of the young animal is not an infallible index of the potential sexuality of the fully mature individual.

While all grades of bisexuality are found in the gonads, self-fertilization is not usual, for the sequence of male and female phases is so regulated that most of the spermatozoa of the initial male phase are discharged before the ova are ready for fertilization. Normally an interval of at least several days intervenes between the two sexual phases. Sometimes a small number of ripe ova may be formed before spermatogenesis is completed, but no case has yet been observed in which the gills contained embryos previous to the discharge of all the sperm.

Development of Gonad

In its early development, the gonad of *Teredo* is not greatly different from that described for other pelecypods (Coe, 1932*a*, 1932*b*), with a branching system of tubular lobes or follicles, each of which is provided with a cortical layer of gonia, while the lumen is filled with proliferating spermatogenic cells (Fig. 1).

The cortical layer, which is usually well demarcated in later stages of development, remains after the spermatogenesis of the preliminary male phase is completed to form the residual cells. The latter, as stated above, have already become differentiated as ovocytes in the protandric females (Figs. 2, 3), but most of them furnish spermatogenic cells in the few true males that accompany their numerous sisters.

Protandric Females

In the early part of the preliminary male phase it appears to be impossible to distinguish sharply between the protandric females and the true males and in both types some of the cells of the cortical layer become differentiated as ovocytes (Plates I and II, Figs. 7–12). In the former the proportion of ovocytes is greater and they increase in size earlier, while in the true males the ovocytes remain small and the spermatogenic cells in the lumens proliferate rapidly. As spermatogenesis continues and the number of spermatozoa increases, additional ovocytes grow in size and begin the deposition of yolk (Fig. 2). With the completion of spermatogenesis the ovocytes are usually about half grown (Plate I, Fig. 9) and after the discharge of the spermatozoa the animal has all the characteristics of a female. Not infrequently some spermatozoa are retained during the early female phase (Fig. 3), and it is not improbable that such may be present when the first ova are released into the mantle cavity. Self-fertilization might then be possible, although normally the eggs are fertilized by sperm from other individuals. The fertilized eggs, as already stated, are retained in the gill chambers until the larval stage is well advanced.

The male phase may be passed so quickly that larvæ metamorphosing in June have reached the female phase and produced a new brood of larvæ by the middle of August. In this brief period of eight to nine weeks the body may attain a length of 80 mm. or more and a diameter of 7 mm. under favorable conditions.

In young animals which complete the initial male phase in the autumn the female phase will be developed during the winter and retained the following summer. It is thought that few if any individuals survive two winters after sexual maturity and there is no evidence that a second sex reversal may ever occur after spawning has been completed at the end of the first breeding season.

There is often a fairly close correlation between the stage of spermatogenesis in the protandric females and the growth of ovocytes in the cortical layer of cells. In young animals during the summer at least a few spermatozoa may be formed before the deposition of yolk commences in most of the ovocytes. But if the gonad develops in the late autumn spermatogenesis is inhibited and the ovocytes continue their growth during the winter and early spring. The large ovocytes then form a complete cortical layer, pushing the spermatogenic cells into the lumens of the follicles (Fig. 5; Plate I, Fig. 7; Plate II, Fig. 10).

In these females there is always more or less degeneration among the spermatogonia, apparently due to the absorption of all the available nutritive materials by the rapidly growing ovocytes. In some cases the entire mass of spermatogenic cells seems to degenerate, so that the preliminary male phase may be abortive, and it is not improbable that an occasional individual may function as a female at its first reproductive period. The conditions in such cases are somewhat similar to those described for the American oyster, where an abortive male phase precedes the functional female phase in all individuals which spawn as females in their first breeding season (Coe, 1932*b*).

True Males

In the second type of gonad the preliminary male phase is of longer duration and the crop of spermatozoa is much larger. In these individuals most of the residual cells appear to be potentially spermatogonia, indicating that the succeeding sexual phase will likewise be male. A few small ovocytes are invariably present, however, (Plate I, Fig. 8) and it is not improbable that a later sex reversal may sometimes occur.

Serial sections of the gonads of a group of functional males show that all phases of bisexuality occur. These lead in a graded series from true males to weakly feminine and strongly feminine hermaphroditic male and protandric female phases. A single collection which was made early in the breeding season (June 24–28) at Woods Hole will serve as an example. Of the 116 individuals taken from piles, 46 were found to be in the functional male phases, 47 were ripe females, and

23 were females bearing embryos. The sex ratio was thus 66 functional males to 100 females. Sections were made of the gonads of 36 of the 46 males; 7 were obviously hermaphrodites, containing ripe sperm and nearly mature ova; 17 were strongly feminine hermaphroditic males, with half-grown ovocytes; 4 males had smaller and fewer ovocytes (weakly feminine); while only 8, or about 26 per cent of the animals with ripe sperm could be classed as true males. Most of the others presumably represent transition stages in the development of the definitive female phase of sexuality. Another collection contained 13 true males, or 30 per cent of the 43 functional males of which the gonads were sectioned. Both were in agreement as indicating a ratio of 10 to 11 true males to 100 functional females in the early part of the breeding season.

Since most or all of the hermaphroditic males would have reached the female phase by midsummer, the population, if no new young had been added and there had been no differential mortality, would presumably have shown at that time only 8.6[1] males to 100 females, a proportion close to that which Quatrefages reported for *T. norvegica* in 1849. The proportion would obviously be changed by later reversals in either direction of the sex tendency indicated by the gonads at the time of examination.

The true males develop more slowly than the other sexual types and consequently their sperm are available for the fertilization of the first crop of ova produced by their more rapidly maturing protandric sisters.

Hermaphroditic Males

In addition to the obviously protandric females and the so-called true males many young individuals are found, both in the summer broods and in the overwintering young adults, which appear to be of an intermediate sexual nature. These are characterized by having only a few ovocytes in the cortical layer (Fig. 6; Plate II, Fig. 12). Such hermaphrodites may produce a small number of ripe ova immediately after spermatogenesis has been completed. They form a graded series in which the expression of the initial male phase has been less definite or the transition from male to female phase less precise than usual.

Under favorable conditions shipworms grow with great rapidity during the warmer portion of the year and the wood may be completely riddled by the borers before any of them have attained the female phase. Heavy infestation and crowded conditions may cause not only dwarfed individuals but not infrequently the early death of all.

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[1] Actual numbers found August 18–21, 1933, were 18 males to 202 females—a surprisingly close approximation to the predicted ratio.

Change in Sex Ratios During the Breeding Season

Because of the protandric nature of the teredo there will obviously be a progressive change in the sex ratios of the population from maleness to femaleness as the breeding season advances. In a population of overwintering young adults the vast majority will be found to be in the initial male phase in late winter and early spring, while the proportions of the two sexes will be reversed after the male phase has been completed and the spawning period is well advanced.

An examination of the teredos in boards taken from a framework in the Eel Pond at Woods Hole May 19, 1933, showed 138 individuals in which active spermatogenesis was in progress and 30 which were classed as females because of the presence of ripe ova or of embryos in the gill chambers. Hence the ratio of functional males to females at this time was 460 males to 100 females (Table I). Other boards from the same source examined when the first larvæ were ready for liberation (June 7–13) yielded 53 individuals in the male phase and 121 which contained ripe ova or had larvæ in the gill chambers. The sex ratio has now changed from 460 males to 44 males for each hundred females.

Table I

Change in Sex Ratio during Breeding Season

Date	Male phase	Female	Total	Ratio, Male to Female
May 19	138	30	168	460 : 100
June 7–13	53	121	174	44 : 100
June 20	15	31*	46	48 : 100
June 16†	23	42	65	55 : 100
June 24–26†	46	70	116	66 : 100

* Most of the large females had died during the preceding week.
† From a different source.

Histological study of the gonads of over two hundred individuals is in full accord with the observations on the living animals. Most of the functional males actually represent the male phase of protandric females as is proved from their gonads when cut in serial sections. Sections of 26 of the 138 individuals determined as functional males from a microscopic examination on May 19 showed that 21 of these were undoubtedly protandric females and only 5 were true males (Table II).

TABLE II

Sexual Phases Preceding First Ovulation

Date	Male phase	Male (Protandric Female)	Total Male	Female	Total
Mar. 29	4	18	22	0	22
Apr. 27	3	9	12	0	12
May 2	1	11	12	0	12
" 16	2	3	5	2	7
" 29	2	3	5	4	9
May 19*	5	21	26	20	46
Sept. 30†	41 (immature)	12	53	1	54

* Selected samples from a different source.

† Estimated age about five to six weeks after metamorphosis.

Since the transition stages from the male to the female phase form a graded series, it is obviously impossible to separate accurately the two sex groups. This is made even more difficult by the fact that a few spermatozoa may remain in the gonads in the early female phase long after spermatogenesis has ceased in most of the follicles, as well as by the presence of spermatozoa from other individuals which tend to accumulate in the mantle cavities of ripe females. As an arbitrary rule the animal has been classed as a male if any considerable number of spermatozoa remain in the gonads, even if there are fully grown ova also. The difference of a single day may thus change the classification of certain individuals.

Later in the breeding season (June 20), after the first crops of larvæ had been liberated, some animals could still be obtained from the same source but most of the large females in the original population had died in the meantime. In spite of this selective death rate, other individuals had reached the female phase to make the ratio of 48 males to 100 females. Instead of more than four functional males to each female, as was the case at the beginning of the breeding season, there was now less than one male to two females. Material from another locality was in close agreement (Table I). Grave (1928) found a ratio of 42.5 males to 100 females in July and early August.

On August 20, 1933, a collection of 220 of the largest, and evidently oldest, individuals from boards placed in the water June 10 contained 18 males, 63 transition females, 82 ripe females, and 57 females bearing larvæ. The sex ratio was therefore 9 males to 100 females. But from a lot of more than 500 smaller, and presumably younger, individuals all except about 2 per cent were functional males. About 38 per cent had reached the early transition stage. The 2 per cent were dwarfed females of the older age group.

A similar population at Woods Hole living in thin pieces of lath and consequently dwarfed in size showed a parallel condition. Previous to the breeding season (March 29), a study of the gonads of 22 of these indicated the bisexual nature of each individual, although there was a great range in the relative size and proportion of the cells characteristic of the two sexes (Plates I and II, Figs. 7, 8, 10–12).

Although spermatogenesis had not yet advanced further than the formation of spermatocytes, some of the gonads were distinctly of the female type, with a cortical layer of large ovocytes in each follicle and relatively few of the small cells, which later prove to be spermatogonia, in the lumens. Others were predominantly male in appearance, consisting of masses of spermatogonia with only a few differentiated ovocytes in the cortical layer (Fig. 5). Still more numerous were the intermediate phases which were typically bisexual and could not be classed as predominantly of either sex (Fig. 6), although they were potentially protandric females.

Table II shows the change in proportion of the sexual phases during the two following months as determined from serial sections of the gonads. All were young animals of the previous year and hatched too late to complete the initial male phase before winter. No functional females appeared before the middle of May, when spermatogenesis was completed in a few individuals. The proportion of females increased thereafter. Although the numbers are small, the data agree with other observations which indicate a regular increase in the proportion of adult individuals in the female phase as the season advances.

A piece of wood infested in early summer and examined in the winter or early spring will reveal an entirely different ratio of sexes. Many of the animals in such wood will have completed the initial male phase late in the preceding summer and will consequently appear as females during the winter. Usually, however, a larger number of smaller and younger males will be found in the same block. Such represent later settlers which have not yet completed the protandric phase. A very few large males, representing the true males of the older age group, may also be present.

It probably happens not infrequently that the young males in one timber furnish the spermatozoa which fertilize the ova of the older females in other timbers in the vicinity. The sperm taken in by the female evidently may be retained for some time in the mantle cavity, for in not a few cases swarms of spermatozoa were found in the mantle cavities of fully ripe females. It is conceivable that secretions, including carbon dioxide, within the female's body may retard the movements of the spermatozoa until they are stimulated to more vigorous action by secretions from the ova as the latter come in contact with the sea water in the mantle cavity and their germinal vesicles break down. The eggs are fertilized almost immediately after reaching the mantle cavity. The oviduct is so widely open that water from the mantle cavity may reach the terminal chambers of the ovary and cause the formation of polar spindles in some of the ova before they leave the oviduct.

In the middle of summer the proportion of the sexes will be entirely different in wood that contains only the overwintered population than it is in pieces recently infested. Selection either by age or by size will then yield groups which are nearly all of one sexual phase or the other. This will account for the great disparity of the sexes noted so often by previous investigators.

In random selection from a general population there will be a great excess of males in the early part of the season, due, as stated above, to the predominance of young individuals in the initial male phase. The proportions will then change gradually to a similar excess of females, as soon as spermatogenesis is completed and the sexual phase changes. Later there will again be an excess of males as the new crop of young becomes sexually mature in July. A less marked influence on the sex ratios may possibly result from environmental conditions.

Conclusion

In comparing the sexual phases of the teredo with those of other animals and particularly with other pelecypods, there seems to be a closer similarity to those of various species of oysters than to any other forms. Protandry is of frequent occurrence in molluscs of widely divergent groups but in none of these is there such an enormous excess of females in the adult population as is here reported for the teredo.

In the teredo the great majority of individuals experience one change of sex. The genetic factors involved in the sex-determining mechanism appear to be so adjusted that the internal environment, not improbably of hormonal nature, normally stimulates the activity of the medullary cells of the follicles during immaturity, leading to the initial male phase of sexuality. Thereafter the cortical cells usually become activated and the animal functions as a female. The external environment may supplement the action of these genetic factors in determining the extent to which the male phase is expressed. This may lead to the observed graded series of hermaphroditic males.

There still remain for consideration the relatively few individuals which have been called true males because they retain the initial male phase for a much longer time than the others and perhaps throughout life. Similar males occur in both larviparous and oviparous oysters, as well as in other groups. It seems probable that they may be dependent upon particular combinations of genetic factors, influenced by physiological (metabolic) conditions during the period when the sexual phase becomes differentiated. The graded series of sexual forms leading from these true males through weakly to strongly feminine protandric females supports the well-known quantitative theories of sex as formulated by Goldschmidt and later by Castle. But it must be emphasized that the series is dependent not only upon the initial sex factors but also upon an age factor, since the female phase appears only after an initial male phase.

In the developing gonad there seems to be little, if any, antagonism between the germinal cells characteristic of the two sexes, for the ovocytes continue their growth, though less rapidly, while spermatogenesis is in progress. The observed sexual phases and the anomalous sex ratios can, perhaps, be best explained by assuming the potential bisexuality of all individuals, associated with alternating genetic factors which bring into activity first one phase of sexuality and then the other. In the California oyster the sequence continues year after year (Coe, 1932*a*), while in the American oyster, which sometimes lives to the age of ten years or more, a facultative change of sex may occur during the winter following a season of functional activity in either sexual phase (Coe, 1932*b*). In the teredo, however, death usually ensues after one full season of female sexuality.

The individuals of all species of *Teredo* live in crowded communities and the reproductive period extends over so long a season that the rapidly maturing young of the early broods may reach the functional male phase in time to fertilize the later crops of ova produced by their own mothers or by other individuals which have attained the female phase in the meantime. Such initial males, together with the very small proportion of true males usually present, afford ample provision for the fertilization of the half-million or more eggs which may be produced by each of the females which constitute the vast majority of the fully adult population. The extraordinary disproportion of the sexes is thereby compensated.

SUMMARY

1. *Teredo navalis morsei* in New Haven Harbor and *T. n. novangliae* at Woods Hole are essentially protandric, nearly all females pass-

ing through a preliminary functional male phase before reaching the definitive phase of sexuality.

2. The primary gonad is bisexual in nature, having a cortical layer of potential ovogonia, with spermatogenic cells filling the lumen of each follicle.

3. The gonads of young individuals in early sexual maturity indicate three intergrading but more or less well demarcated types of functional males: (a) initial males, representing the protandric phase of the females; these have a complete cortical layer of ovocytes surrounding the relatively small amount of spermatogenic tissue in the lumens of the follicles; (b) hermaphroditic males; these have relatively few large ova scattered among the spermatogonia of the cortical layer; (c) true males; most of the ovocytes in the cortical layer remain small or degenerate.

3a. In the protandric females the cortical cells become differentiated into ovocytes and begin the deposition of yolk while spermatogenesis is in progress in the lumens. The functional male phase may be reached within six weeks after metamorphosis and is of brief duration. A few spermatozoa may be retained until the first ovulation, after which the animal seems to have exclusively female characteristics.

3b. In hermaphroditic males the functional male phase is of longer duration than in typical protandric females and the number of spermatozoa produced is much greater. A later transformation to the female phase is presumably of usual occurrence, but is not invariable.

3c. True males form a small proportion of the population. Growth continues during a long period of spermatogenesis. After the discharge of the first crop of spermatozoa most of the residual cells still retain their male characteristics and no evidence of a later change of sex has been found, although small ovocytes are usually scattered among the cortical cells.

4. The proportion of individuals in the two functional sexual phases changes with the season, there being a great excess of males at the beginning of the breeding season due to the protandric nature of the young animals in the early period of sexual maturity; there is a corresponding excess of females later in the season, due to the change of sexual phase after spermatogenesis in the initial male phase has been completed. After the end of July the proportion of males may again be greatly in excess if the new crop of young is included. Selection of the larger (and older) individuals will then yield mainly females, while the smaller (and younger) animals may all be in the male phase.

5. In the early part of the breeding season the populations from two localities each consisted of about 33 per cent functional males and 67

per cent mature females. Less than one-third of the functional males were classed as true males, the others indicating the potentiality of a later change to the female phase. By the middle of August only 9 males to 100 females were found in the older age group.

6. After the assumption of the definitive female phase there is no evidence of a second sex reversal.

LITERATURE CITED

BARTSCH, PAUL, 1922. A Monograph of the American Shipworms. *Bull. 122, U. S. Nat. Mus.*, p. 1.

COE, W. R., 1932*a*. Development of the Gonads and Sequence of the Sexual Phases in the California Oyster (Ostrea lurida). *Bull. Scripps Inst. Oceanog., Tech. Ser.*, **3**: 119.

COE, W. R., 1932*b*. Sexual Phases in the American Oyster (Ostrea virginica). *Biol. Bull.*, **63**: 419.

COE, W. R., 1933. Destruction of Mooring Ropes by Teredo; Growth and Habits in an Unusual Environment. *Science*, **77**: 447.

GRAVE, B. H., 1928. Natural History of Shipworm, Teredo navalis, at Woods Hole, Massachusetts. *Biol. Bull.*, **55**: 260.

KOFOID, C. A., AND R. C. MILLER, 1927. Biological Aspects of the Marine Borer Problem. *Final Rep. San Francisco Bay Marine Piling Com.*, p. 188.

MAY, E., 1929. Beiträge zur Kenntnis der Hartteile der Terediniden. (Moll. Lamellibr.) *Zeitschr. Morph. u. Ökol. d. Tiere*, **15**: 559.

NELSON, T. C., 1922. The European Pileworm; a Dangerous Marine Borer in Barnegat Bay, New Jersey. *N. J. Agr. Exp. Sta., Circ.*, **139**: 1.

NELSON, T. C., 1928. On the Distribution of Critical Temperatures for Spawning and for Ciliary Activity in Bivalve Molluscs. *Science*, **67**: 220.

SIGERFOOS, C. P., 1908. Natural History, Organization, and Late Development of the Teredinidae, or Ship-worms. *Bull. U. S. Bur. Fish.*, **27**: 193.

YONGE, C. M., 1926. Protandry in Teredo norvegica. *Quart. Jour. Micr. Sci.*, **70**: 391.

STUDIES ON AMPHIBIAN METAMORPHOSIS. XI. DEVELOPMENT AND REGRESSION OF DERMAL PLICÆ FOLLOWING HOMOPLASTIC AND HETEROPLASTIC TRANSPLANTATION

O. M. HELFF

Biology Department, University College, New York University

Introduction[1]

The writer (1931) has previously pictured and described the normal histological developments of dermal plicæ structures as they occur during the metamorphosis of *Rana pipiens* larvæ. Reciprocal, autoplastic transplantations were also made between potential lateral and dorso-lateral dermal plicæ integument and integument of the back and belly. During involution it was observed that the back and belly integumentary transplants never developed dermal plicæ structure, although directly adjacent to normally developing dermal plicæ. The potential dermal plicæ integument, previously transplanted to the back or belly, however, always underwent a normal development resulting in fully-formed dermal plicæ by the close of larval involution. It was concluded, therefore, that certain areas of integument become specific for dermal plicæ development before the onset of larval involution, and that such development is the result solely of influences present in the blood-stream during metamorphosis. Apparently, the location of the potential dermal plicæ integument in no way influences its later histological developments.

As a result of the above described work, several interesting problems presented themselves which may be briefly stated in the following questions:

1. Is dermal plicæ development possible following heteroplastic transplantation?
2. Is the stimulus for dermal plicæ development still present following involution of the larva?
3. Can mature dermal plicæ integument maintain its histological structure when transplanted to a non-metamorphosing host or is its stability dependent on influences present only in the metamorphosing or fully-metamorphosed animal?

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[1] Since no extended discussion concerning the various problems relating to amphibian dermal plicæ and integumentary glands is intended in the present paper, the reader is referred to the bibliographies of Dawson (1920), Helff (1931), and Weiss (1908), (1915).

The present paper concerns the results of transplantation experiments made in an attempt to answer the above questions. The following series of transplantations were made:

1. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. catesbeiana* and *R. palustris* larvæ.
2. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. catesbeiana* and *R. clamitans* larvæ.
3. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. palustris* and *R. clamitans* larvæ.
4. Homoplastic transplantation of potential dermal plicæ integument of *R. palustris* larvæ to newly metamorphosed *R. palustris* frogs.
5. Heteroplastic transplantation of potential dermal plicæ integument of *R. catesbeiana* larvæ to newly-metamorphosed *R. palustris* frogs.
6. Homoplastic transplantation of differentiated dermal plicæ from newly-metamorphosed *R. palustris* frogs to *R. palustris* larvæ.
7. Heteroplastic transplantation of differentiated dermal plicæ from newly-metamorphosed *R. palustris* frogs to *R. catesbeiana* larvæ.

RESULTS

Heteroplastic Transplantation of Potential Dermal Plicæ Integument

Three species of anuran larvæ were selected for the heteroplastic transplantation work, namely: *R. catesbeiana, R. clamitans,* and *R. palustris.* They were collected in the vicinity of Lake Winnisquam, New Hampshire, during the summer of 1932 in large numbers, making selection of various larval stages quite feasible. The *R. palustris* larvæ varied in length from 67 to 80 mm., with hind-limbs 5 to 35 mm. long; the *R. clamitans* larvæ being from 64 to 83 mm. in length, with hind-limbs 7 to 30 mm. long; while the *R. catesbeiana* larvæ were large, second-year forms 112 to 116 mm. long, with hind-limbs 9 to 15 mm. in length. In no case were larvæ used, however, in which histological signs of dermal plicæ structures had developed.

The technique of transplantation employed was similar to that as previously described by the writer (1931). This may be briefly stated as follows: Following chloretonization of the larvæ, a rectangular piece of integument was removed from the potential dorso-lateral dermal plicæ region of one individual and transplanted, heteroplastically, to the same region of another. Upon recovery from the anæsthetic, the larvæ were isolated in individual aquaria and observations of the grafts made during the subsequent metamorphosis of the host animals. In the case of *R. catesbeiana* larvæ, it was necessary to feed desiccated thyroid in order to induce complete involution. The *R. clamitans* and

R. palustris larvæ, however, usually underwent normal transformation. Following metamorphosis of the host animals, the transplants were removed for histological identification and examination of dermal plicæ structures.

Twenty-eight cases of complete metamorphosis were obtained using *R. palustris* larvæ to which *R. catesbeiana* integument had previously been transplanted. The time required for the attainment of complete larval involution (the time the transplants remained on the hosts) varied between 27 and 53 days. The dermal plicæ of the host animals developed normally and were well formed by the time complete involution of the latter occurred. The transplanted *R. catesbeiana* grafts, however, underwent a definite sequence of changes. The transplants healed readily and appeared quite normal for several days following grafting. Following this period, a general darkening of the grafts became progressively more evident; the transplants being quite black by the time complete involution of the host was obtained. A marked reduction in surface area of the transplants (from 30 to 50 per cent) also took place. In many cases definite erosion of the epithelium was also noticed. In the majority of cases there were no external signs of dermal plicæ formation, although in a few there were evidences of a faint light-colored stripe typical of early plicæ formation.

The histological examination consisted of serial sectioning of transplants together with a small amount of the adjacent dermal plicæ integument of the host. The skin of the host invariably showed normal, well-developed dermal plicæ structures. As for the transplants, no thickening of integumentary layers was ever observed. There was, however, definite evidence in several cases of a slight development of the mucous glands (see Fig. 1), characteristic of early dermal plicæ formation. That considerable degeneration of the transplants had taken

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EXPLANATION OF PLATE I

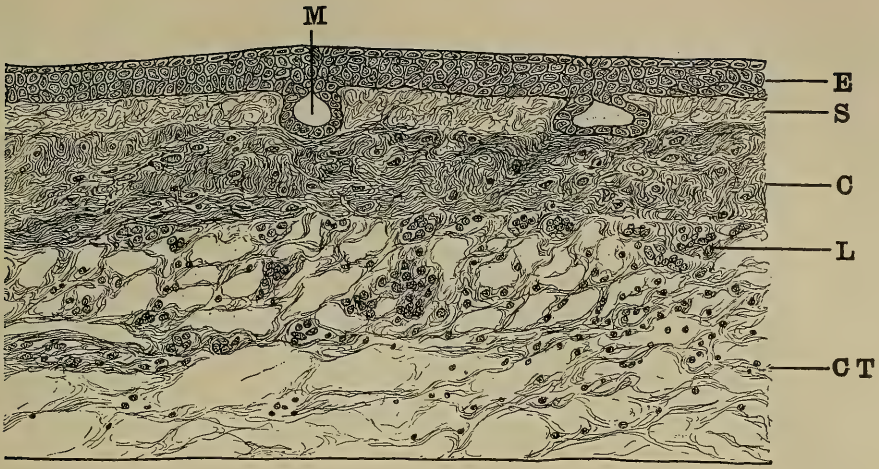
FIGS. 1 to 3. *BV,* blood vessel; *C,* stratum compactum; *CT,* subcutaneous connective tissue; *D,* duct of mucous gland; *E,* epidermis; *L,* lymphocytes; *M,* mucous gland; *P,* poison gland; *S,* stratum spongiosum; *X,* junction of transplant and host integuments, transplant to the left of *X,* host integument to the right.

FIG. 1. Histological section through *R. catesbeiana* integument on *R. palustris,* showing slight development of mucous glands and generalized histolysis.

FIG. 2. Histological section through *R. catesbeiana* integument on *R. clamitans* and adjacent integument of the latter. Note glandular development in host skin and absence of same coupled with pronounced histolysis in the transplant integument.

FIG. 3. Histological section through *R. palustris* dermal plicæ three weeks following transplantation to non-metamorphosing *R. palustris* larva. Note normal appearance of glandular structures and integumentary layers.

Plate I



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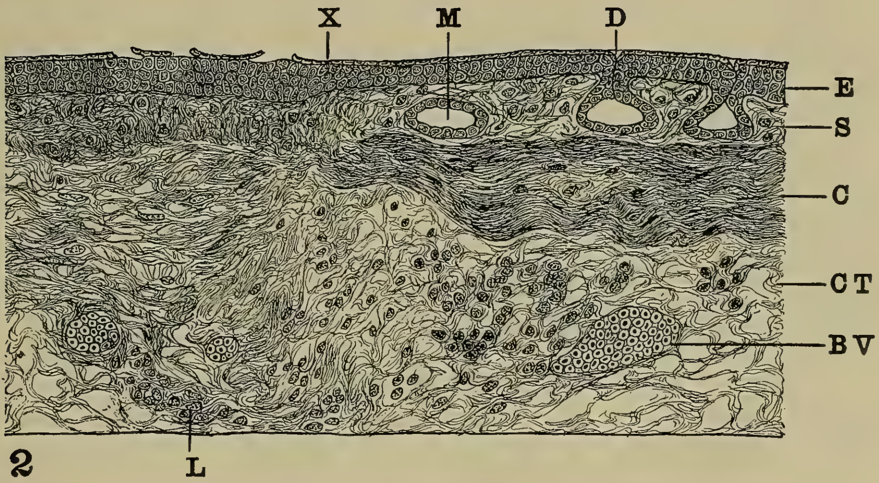
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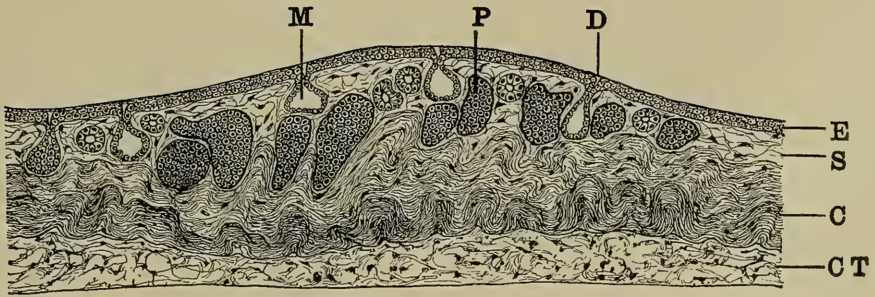

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place was evident by the large number of lymphocytes present in the subcutaneous connective tissue and the invasion of the stratum compactum by these cells.

The reciprocal transplantation of *R. palustris* integument to *R. catesbeiana* larvæ gave quite similar results. Twenty-four cases of complete host metamorphosis were obtained, the normal dermal plicæ of which were always well developed. The *R. palustris* transplants underwent progressive darkening and reduction in surface area, while no external signs of dermal plicæ formation were ever observed. Histological examination likewise failed to show even slight signs of glandular development or the formation of other dermal plicæ structures. Evidences of tissue histolysis and lymphocytic action were not as pronounced, however, as had been observed for the *R. catesbeiana* transplants.

Twenty-three cases of complete metamorphosis were obtained of *R. clamitans* larvæ to which *R. catesbeiana* potential dermal plicæ integument had previously been transplanted. The normal dermal plicæ of the host animals were undergoing development at the close of the involution period, although no external signs of such development were present in the transplants; which at this time had been on the involuting larvæ from 23 to 31 days. The latter also became quite black in coloration and reduced in surface area from 40 to 60 per cent. Histological examination gave evidence of early plicæ formation in the adjacent host skin, but none for the transplanted integument. Very pronounced signs of histolysis were present in the latter, resulting in complete disintegration, in many cases, of the stratum compactum layer and the associated infiltration of phagocytic lymphocytes (see Fig. 2).

Series of transplantations were also made of *R. clamitans* potential dermal plicæ integument to *R. catesbeiana* and *R. palustris* larvæ and of *R. palustris* integument to *R. clamitans* larvæ. Whether or not dermal plicæ development is possible following these heteroplastic integumentary transplantations was not determined, due to the fact that the host animals invariably died before metamorphosis had progressed far enough for the dermal plicæ of the latter to begin their development. The cause of death was not determined, although the inference might be drawn that the integumentary grafts exercised a toxic effect on the host animals. In all series, however, it was observed that considerable reduction in surface area of the transplants had taken place prior to the death of the host animals.

Transplantation of Potential Dermal Plicæ Integument to Newly-metamorphosed Animals

The formation of normal dermal plicæ structures proceeds steadily during the metamorphosis of the larva, resulting in distinct glandular and cellular developments by the time larval involution is completed. Following metamorphosis, however, there is a continued growth and development of the various dermal plicæ components accompanying the growth of the young frog. Whether these later developments are the result of influences still present in the metamorphosed animal, or whether the developments are simply the end results of earlier larval stimuli is an interesting point in question. Transplantation of potential dermal plicæ integument from the larva to the young frog should serve to answer these questions, since development of dermal plicæ structures under such conditions would indicate a continued influence on the part of the host, while failure of such development would serve to justify the reverse conclusion. The results of such transplantations are described below.

Thirty-six transplantations were made from *R. palustris* larvæ to newly-metamorphosed *R. palustris* frogs. The larvæ varied in length from 60 to 78 mm., with hind limbs 1 to 19 mm. long, while the young frogs were normal in all respects except for the persistence of small tail stumps 2 to 15 mm. in length. In making the transplantations, rectangular areas of integument from the dorso-lateral dermal plicæ regions were first removed from the young frogs and the potential dermal plicæ skin from the larvæ was then transplanted to these wound areas. The young frogs lived from 20 to 27 days following such transplantations, at which time the grafts were removed for fixation and subsequent histological examination.

In the preponderence of cases, a definite thinning of the transplants occurred, which was subsequently shown to be due to a progressive cellular histolysis of the various integumentary layers. In several cases, resorption of the grafts was so pronounced that large central areas were entirely eroded away. In such cases the histolytic processes were so intense as to affect the structure of the adjacent dermal plicæ of the host animal (see Fig. 5). Several cases of the latter condition were observed in which mucous and poison glands had undergone nearly complete degeneration, while the various integumentary layers, with the exception of the epidermis, were markedly affected. Three cases presented relatively little cellular degeneration of the transplanted integument. These gave external evidence of the formation of faint white-colored stripes, characteristic of early dermal plicæ formation. Upon sectioning, slight glandular development was evident, which, however, never progressed to the extent of producing thickening of the stratum spongiosum.

Heteroplastic transplantations were also made from *R. catesbeiana* larvæ to young *R. palustris* frogs. The larvæ varied in length from 86 to 116 mm., with hind limbs 2 to 11 mm. in length, while the *R. palustris* frogs were similar to those used for the homoplastic transplantations. Of the twenty-eight transplantations made, twenty-six of the young frogs lived from 18 to 25 days following the operative period, while two lived for 37 and 39 days, respectively.

The *R. catesbeiana* integumentary grafts invariably assumed a darker coloration as compared with the adjacent host skin. There were also definite signs, in many, of epidermal erosion. In the majority of cases there were no external signs of dermal plicæ formation, although slight evidences of glandular development were usually seen upon sectioning. In several individuals (including the two cases living for 37 and 39 days following the transplantation period) definite ridges were formed. These ridges were quite characteristic of early dermal plicæ formation, although whitening of the ridge did not occur. Sectioning revealed pronounced development of mucous and poison glands with considerable hypertrophy of the stratum spongiosum (see Fig. 4). Even in these cases, however, considerable evidence of tissue histolysis was evident.

Transplantation of Well-developed Dermal Plicæ to Non-metamorphosing Larvæ

The stability of well-formed dermal plicæ structures, and their possible ability to continue growth when removed from influences of the metamorphosed animal, was determined by transplantation of dermal plicæ from newly-metamorphosed frogs to non-metamorphosing larvæ. Should such transplants maintain normal plicæ structures, the inference could be drawn that their stability is not dependent on influences prev-

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EXPLANATION OF PLATE II

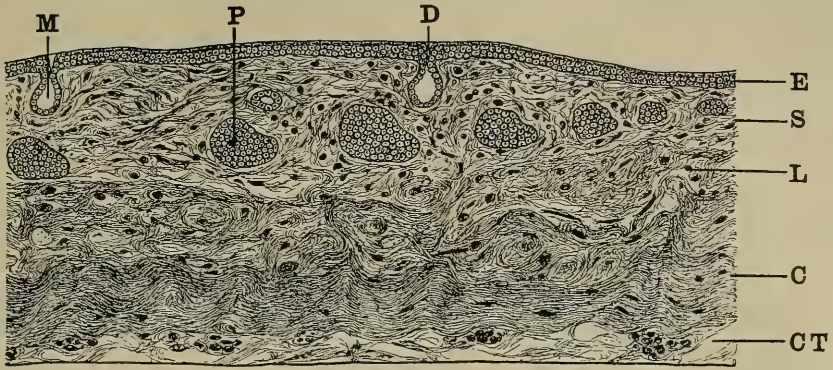
FIGS. 4 to 6. *BV,* blood vessel; *C,* stratum compactum; *CT,* subcutaneous connective tissue; *D,* duct of mucous gland; *DG,* degenerating poison gland; *E,* epidermis; *L,* lymphocytes; *M,* mucous gland; *P,* poison gland; *S,* stratum spongiosum; *X,* junction of transplant and host integuments, transplant to the left of *X,* host integument to the right.

FIG. 4. Histological section through *R. catesbeiana* integument on young *R. palustris* frog 37 days following transplantation. Note development of mucous and poison glands and dissociation of upper layers of stratum compactum.

FIG. 5. Histological section through *R. palustris* integument on young *R. palustris* frog 25 days following transplantation. Note pronounced histolysis of transplant and histolytic effect on adjacent integument of the host.

FIG. 6. Histological section through *R. palustris* dermal plicæ three weeks following transplantation to non-metamorphosing *R. catesbeiana* larvæ. Note absence of glandular structures and infiltration of large lymphocytes.

Plate II



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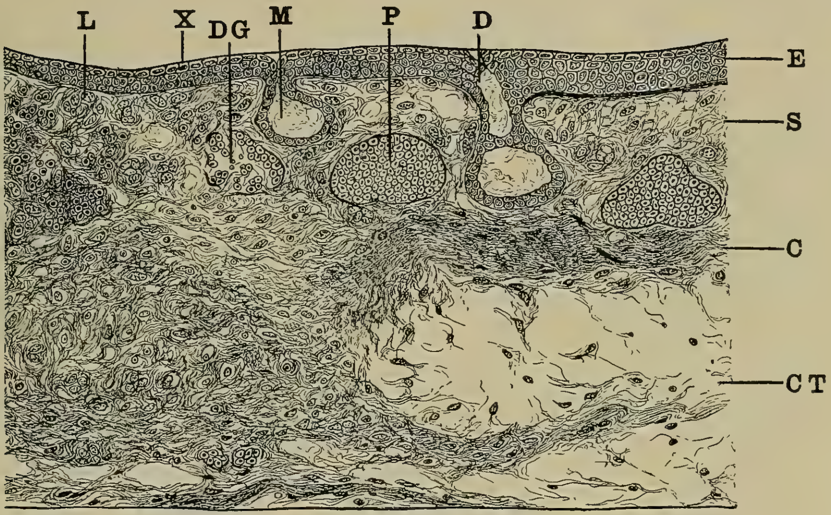
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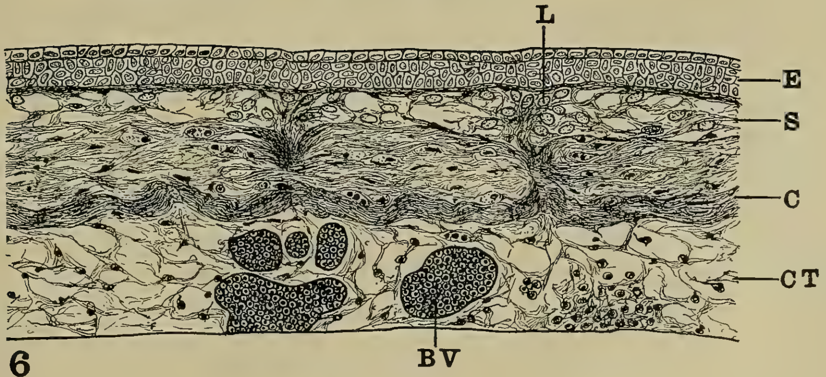

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alent in the young frog. Should regression of structures occur, the reverse conclusion could be proposed. The results of such transplantations are described below.

Sixteen transplantations were made of dorso-lateral dermal plicæ integument from newly-metamorphosed *R. palustris* frogs to R. *palustris* larvæ. The young frogs were completely metamorphosed with the exception of small tail stumps 5 to 11 mm. in length. The dermal plicæ of such individuals was well developed and appeared externally as a white, definitely ridged structure. Histologically, the plicæ were characterized by well-formed mucous and exceptionally large poison glands. The host larvæ were selected on the basis of small hind-limb growth, representing a stage of development in which metamorphosis would not begin for at least five or six weeks. The hind limbs of such individuals varied from 2 to 6 mm. in length, while the total length varied between 61 and 69 mm.

In general, the transplants underwent a gradual change in coloration from their original greenish hue to the almost black shade of the host integument. There was no evidence of a reduction in surface area, while the dermal plicæ underwent no external diminution either as regards size or visibility. Twenty-one days following transplantation, the fourteen larvæ that had survived were killed and the transplants removed for sectioning and subsequent study. Histological examination gave evidence that no reduction of dermal plicæ glands or integumentary layers had occurred. The histological appearance was practically the same as when transplanted (see Fig. 3). It should be noted here, however, that development of dermal plicæ structures never progressed beyond the stage already attained when previously removed for transplantation. Normally, on the newly-metamorphosed frog, a definite enlargement of dermal plicæ structures takes place within a three-weeks interval following larval involution.

A second series of transplantations involved the grafting of similar dermal plicæ integument from newly-metamorphosed *R. palustris* frogs onto non-metamorphosing *R. catesbeiana* larvæ. The donors in this case were in the same stage of development as described for the homoplastic transplantations, while the *R. catesbeiana* larvæ were second-year forms from 91 to 116 mm. in total length with hind limb buds 2 to 11 mm. in length. Such larvæ were not destined to metamorphose until the following summer.

Twenty transplantations were made in all. Fourteen of the host animals survived for three weeks, at which time the transplants were removed for sectioning. During the three-weeks interval on the host larvæ, the grafts underwent practically no reduction in area, although a generalized darkening occurred until the coloration matched that of the adjacent larval integument. A very definite external diminution in the appearance of the dermal plicæ stripe took place, however, resulting in several cases in the complete disappearance of all macroscopic signs of the latter. Where the dermal plicæ stripe was still visible, it was usually much darker in coloration as compared with its original appearance. Histological examination of sections showed that a progressive histolysis of glandular structures, in particular, had taken place. Where the stripe was still visible, externally, the glands were found to be much reduced in size and undergoing degeneration. Where no external signs were apparent, usually all vestiges of glandular formations had disappeared (see Fig. 6). The lymphocytes were particularly abundant in such cases, while remnants of histolyzed structures could frequently be detected as far down as the subcutaneous connective tissue.

Discussion

The results of heteroplastic transplantations between larvæ indicate that while the grafts appear to heal and remain healthy for a time, cellular histolysis always occurs during the involution of the host animal. In this respect *R. catesbeiana* integument would appear to be less resistant than *R. palustris* integument, following reciprocal transplantation. Again, *R. catesbeiana* integument proved to be much less resistant to histolysis when transplanted to *R. clamitans* than when grafted to *R. palustris*. Obviously, the histolysis of heteroplastic integumentary transplants must be concerned with species specificity.

In general, it may be said that the failure of dermal plicæ to develop following such heteroplastic transplantations, was correlated with and probably due to inhibiting influences set up by the histolytic reactions. In this respect it should be noted that glandular development occurred only in cases where relatively little histolysis ensued, *i.e.,* in several transplantations of *R. catesbeiana* integument to *R. palustris* larvæ. It is interesting to note here, also, that due to the more rapid involution of *R. palustris* larvæ as compared with *R. catesbeiana* and *R. clamitans* larvæ, the dermal plicæ of the former species normally develop at a faster rate. This in turn would seem to indicate that the influences which stimulate dermal plicæ formation in *R. palustris* are more intense than is true of those present in the other two species. This difference, if true, may account for the development of glandular elements in *R. catesbeiana* integument transplanted to *R. palustris*. The results in general, however, indicate that dermal plicæ development is possible following heteroplastic transplantation, providing the integumentary transplants do not undergo pronounced cellular histolysis.

The results of integumentary transplants made to young *R. palustris* frogs show quite conclusively that dermal plicæ development is possible in such cases. The fact that such development was more pronounced in *R. catesbeiana* integument than in *R. palustris* integument transplanted to *R. palustris* frogs calls for interpretation since one would expect the reverse to be the case. If we assume, however, as seems quite likely, that *R. catesbeiana* dermal plicæ are normally subjected to a less intense developmental stimulus as compared with the normal dermal plicæ of *R. palustris,* then development of the former should be more easily elicited as compared with that of the latter when under the more intense stimulating influence of *R. palustris.* Whether or not the developmental differences, as recorded, can be explained in this manner, the results in general indicate that post-involution growth and differentiation of dermal plicæ structures is due to a continued developmental influence on the part of the growing frog.

Transplantation of well-formed dermal plicæ to non-metamorphosing larvæ gave evidence of the stability of glandular and integumentary layers during the period of the experiment (21 days). This was true, however, only for the homoplastic transplantations. The pronounced, and in some cases total, degeneration of dermal plicæ structures following heteroplastic transplantation was due, no doubt, to the general histolysis of the transplants which occurred. It must be admitted, however, that in some cases the degree of general histolysis did not justify the pronounced dermal plicæ degeneration observed. One striking fact regarding the homoplastic transplantations was that no further growth or development of dermal plicæ structures had taken place during the three weeks interval on the larva, while the normal dermal plicæ of the donors had undergone considerable enlargement during the same time interval. These results, therefore, again emphasize the conclusion that dermal plicæ of the metamorphosed animal require and continue to respond to developmental influences present in the growing frog.

The various developments and regressions of dermal plicæ structures as described in the present paper are provocative of interesting possibilities concerning the persistence of potentialities for dermal plicæ formation. Since total regression is possible following heteroplastic transplantation, it would be interesting to see whether such transplants could again develop dermal plicæ structures in a suitable environment. Since the writer (1928) has previously shown that any integument placed over the annular tympanic cartilage will develop into tympanic membrane during larval involution, it would be of interest to determine whether integument having first undergone complete dermal plicæ regression could secondarily develop into tympanic membrane upon suitable transplantation. The above and related questions are at present under investigation by the writer.

Summary and Conclusions

Heteroplastic transplantation of potential dermal plicæ integument was made between larvæ of three species to ascertain possible development of dermal plicæ structure during host metamorphosis. Results were as follows:

R. catesbeiana to *R. palustris*—slight development.

R. catesbeiana to *R. clamitans*—no development; grafts underwent considerable resorption.

R. palustris to *R. catesbeiana*—no development; slight resorption of grafts.

Homoplastic and heteroplastic transplantations of potential dermal plicæ integument were made between larvæ and newly-metamorphosed adults to determine whether the stimulus for dermal plicæ development is retained following involution. Results were as follows:

R. palustris to *R. palustris*—pronounced resorption of grafts in most cases; slight development in several cases where little resorption occurred.

R. catesbeiana to *R. palustris*—in general, pronounced development, especially of mucous and poison glands.

Homoplastic and heteroplastic transplantations of well-developed dermal plicæ integument were made from newly-metamorphosed frogs to non-metamorphosing larvæ to determine the stability of dermal plicæ structure or its possible regression. Abbreviated results were as follows:

R. palustris to *R. palustris*—no regression; typical structure maintained.

R. palustris to *R. catesbeiana*—very definite regression; no histological characteristics remaining in several cases.

In general, it may be concluded that development is possible following heteroplastic transplantation providing pronounced integumentary histolysis does not occur. Post-involution growth and differentiation of dermal plicæ structures is due to continued developmental influences present in the growing frog. *R. palustris* dermal plicæ resist regression following homoplastic transplantation but are susceptible to regression following heteroplastic transplantation to non-involuting larvæ.

LITERATURE CITED

Dawson, A. B., 1920. The Integument of Necturus maculosus. *Jour. Morph.*, **34**: 487.

Helff, O. M., 1928. Studies on Amphibian Metamorphosis. III. The influence of the annular tympanic cartilage on the formation of the tympanic membrane. *Physiol. Zool.*, **1**: 463.

Helff, O. M., 1931. Studies on Amphibian Metamorphosis. IX. Integumentary specificity and dermal plicæ formation in the anuran, Rana pipiens. *Biol. Bull.*, **60**: 11.

Weiss, O., 1908. Ueber die Entwickelung der Giftdrüsen in der Anurenhaut. *Anat. Anzeig.*, **33**: 124.

Weiss, O., 1915. Zur Histologie der Anurenhaut. *Arch. f. mikr. Anat.*, **87**: 265.

OBSERVATIONS ON THE PENETRATION OF DAYLIGHT INTO MID-ATLANTIC AND COASTAL WATERS

GEORGE L. CLARKE

(From the Woods Hole Oceanographic Institution,[1] Woods Hole, Massachusetts; and the Laboratory of General Physiology, Harvard University, Cambridge, Massachusetts)

Although considerable information on the penetration of daylight into the sea has been obtained from measurements made by Shelford and Gail (1922), Shelford (1929), Poole and Atkins (1929), Atkins (1932), and Atkins and Poole (1933), it was desired to extend this type of investigation to other regions and to study in further detail the effect of varying conditions upon submarine irradiation.[2] It was hoped to make observations in the open ocean, where the photo-electric method had never before been employed, and to compare the intensity of irradiation found there with that existing in coastal regions. As far as possible it was desired to learn to what extent the degree of light penetration is modified by changes in latitude, in time of day, and in condition of the sea surface. This information is of particular importance in relation to the theories for the vertical distribution of plankton (cf. Russell, 1927; 1931). We therefore proposed to measure the intensity of submarine irradiation at the same time that plankton hauls were being made. The application of our results to the theory of the effect of light on the diurnal migration of plankton is considered in a separate paper (Clarke, 1933).

The observations presented in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution. The photometric apparatus was put aboard at Plymouth, England, in July, 1931. Measurements of the penetration of light into the sea were made at thirteen stations on the trip across the Atlantic during the succeeding six weeks. The work was continued in July and August, 1932, when several series of observations were carried out in the Gulf of Maine and in Woods Hole Harbor.

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[1] Contribution No. 26.

[2] By irradiation is meant the radiant flux density of all wave lengths (cf. Sawyer, 1933), as distinct from illlumination which refers to visible wave lengths only. The term "radiation" has formerly been used loosely for irradiation.

APPARATUS

The apparatus for the measurement of submarine irradiation assembled for the "Atlantis" has been described in detail by Gall and Atkins (1931). Each photometer contains a potassium (sensitized with hydrogen) photo-electric cell. Two of the cells are of the vacuum type; the third is gas-filled. The photo-electric current is measured with a potentiometer or neon lamp outfit. Essentially, the method consists in measuring alternately the intensity of daylight falling on deck and that penetrating to various depths beneath the surface of the sea. Values are thus obtained for the submarine irradiation as percentages of the light at the surface. Absolute values of the intensity of light are derived by calibration of the instruments against a photometric standard.

With the original arrangement of the apparatus considerable trouble was caused by the fouling of the lines intended to operate the shutter of the submarine photometer and by the horizontal straying of the instrument due to the drift of the ship and the great sideward resistance of the lines and cables. Some improvement was produced by replacing one line by a heavy weight which opened the shutter. However, fouling and straying were still troublesome in rough weather and whenever working at depths greater than 50 meters. Accordingly, the No. 1 photometer was provided with a new four-conductor insulated cable and with an internal shutter which can be opened and closed electrically.

The circuit for the internal shutter uses two of the conductors of the new cable. The other two conductors are used for the photoelectric circuit. The new cable was made for the Woods Hole Oceanographic Institution by the General Electric Company. It is 300 meters long, covered with a very tough rubber jacket, and is capable of withstanding a strain of 168 kilograms without injury. The original five lines running to the photometer from the ship have thus been reduced to two, viz., the steel supporting cable and the new four-conductor cable. Fouling never occurs even in rough weather, and since the diameter of the new cable is only 1.7 cm., the sideward resistance of the whole apparatus is greatly reduced. During the work following these improvements the supporting cable rarely reached an angle with the vertical greater than 20°.

The original external shutter has been left attached to the photometer case for protection of the glass window when not in use. Just before the photometer is swung over the side, this shutter is tied open. The internal device was made from a Packard photographic shutter, the aperture of which is 9 cm. in diameter. The rubber bulb, which ordinarily operated the shutter, has been removed and a short lever arm attached to the post which, when moved to the left or to the right, opens or closes the leaves of the shutter. The lever arm is arranged as the armature of an electro-magnet (see Fig. 1). The free end of the lever arm, which is attached to the shutter, swings to the left or to the right according to whether the left-hand pole or the right-hand pole of the magnet is activated. Each pole is wound with 500 feet of double silk insulated No. 30 copper wire. One side of each coil is grounded to the case (and hence through the sea water and supporting steel cable to the hull of the ship).

The operation of the shutter is controlled by a double throw switch conveniently located in the deck laboratory near the potentiometer. The ship's current is used to activate the coils of the electro-magnet (see Fig. 1). Closing the circuit momentarily suffices to open the shutter, or to close it. The shutter remains in the position in which it is left when the current is cut off. Since the current is allowed to flow for only a short interval, the coils may be greatly overloaded, thus increasing the strength of the magnet. Ordinarily 0.4 ampere is sufficient to operate the shutter but the current may be increased to one ampere if necessary. This method has the additional advantage that no current is flowing through the electro-magnets while measurements in the photo-electric cell are being made.

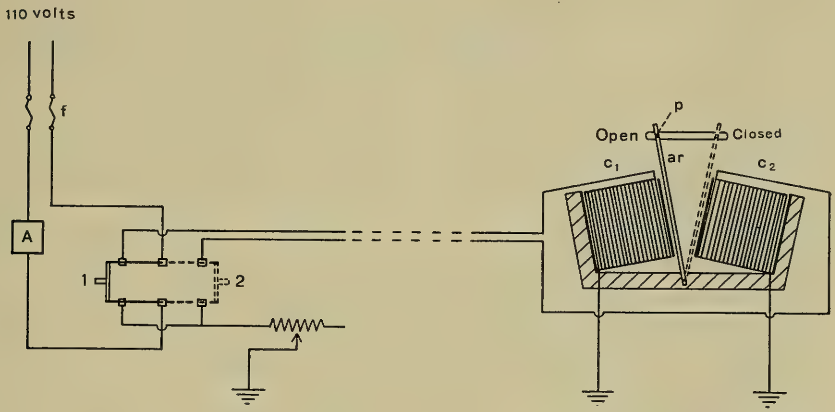

FIG. 1. Diagram of the connections of the electrically operated shutter.
(1) Position of switch to open shutter.
(2) Position of switch to close shutter. Except for the moments of operating the shutter, the switch is left with contacts broken in the vertical position.
A, ammeter; *ar*, armature; c_1, c_2, coils of electromagnet; *f*, 1-ampere fuses; *p*, post attached to leaves of shutter.

Standardization of Photometers

Before the "Atlantis" sailed from Plymouth, the photometers were compared by Dr. W. R. G. Atkins with a vacuum potassium cell which had been standardized previously against an open carbon arc lamp (see Gall and Atkins, 1931, where the lowest and highest intensities of light which can be measured by our apparatus are stated also). The values obtained by him are summarized in Table I.

Birge and Juday (1932, p. 525) have pointed out that the meter-candle is not a suitable unit for the measurement of irradiation under water. This unit is misleading when applied to the visible part of the solar radiation penetrating beneath the surface because the energy spectrum has become so profoundly modified. Moreover, the radiation beyond the limits of the visible should be measured in addition, and this cannot properly be expressed in meter-candles. Whatever unit is used, the following precaution must be taken in order to obtain a correct standardization of the photometer: in evaluating the standard source only that region of its spectral emission may be considered which

Table I

Standardization of photometers. Irradiation necessary to produce photo-electric current of 1 microampere at an anode potential of 60 to 63 volts.

Photometer	Carbon arc used as a standard *	1000-watt Mazda lamp used as a standard		Photo-electric emission produced by test lamp (used to check for changes in sensitivity at sea)
	Irradiation extending over visible range	Irradiation extending over visible range	Irradiation within range: 3460A°–5260A°	
	meter-candles	*meter-candles*	*microwatts/cm.^{2}*†	
Deck	370	1610	135	4.4 μa
No. 1 Sea (1931)	870	2080	175	—
No. 1 Sea (1932)	—	3760	315	2.1
No. 2 Sea	232 ‡	750 ‡	63 ‡	—

* Values given by Gall and Atkins (1931).

† 1 Microwatt/cm.^{2} = 10 ergs/cm.^{2}/sec. = .0000143 gm. cal./cm.^{2}/min.

‡ Values obtained immediately after glowing the cell for one second (see Gall and Atkins, 1931).

It will be observed that the ratios of the carbon arc and the Mazda lamp standardizations are not the same for the three photometers. Evidently the sensitivities of the photo-electric cells changed considerably during the year which elapsed between the tests.

The decreased sensitivity of No. 1 as used in 1932 was caused by the greater distance between the photo-electric cell and the photometer window. This change in position was necessary in order to make room for the new internal shutter.

occurs within the spectral range to which the photometer actually used is sensitive. Measurements made without this precaution give an erroneous idea of the submarine irradiation and the actual values obtained depend upon the type of source used for standardization. An illustration of this is to be found in Table I, where three standardizations of our photometers are compared. The values in meter-candles based upon the carbon arc differ widely from those based upon the incandescent lamp. The discrepancy is due to the fact that the whole visible radiation of the two standards has been measured and that these differ in spectral distribution, particularly in the region to which our photometers are most sensitive (see below).

Submarine irradiation may be properly expressed in cal./cm.^{2}/min., in ergs/cm.^{2}/sec., or in microwatts/cm.^{2}, provided that the spectral range measured is stated. In this paper calibration values are given in microwatts/cm.^{2} for the narrow range within which our photometers are sensitive. To facilitate comparisons between different investigations it is desirable to state the irradiation at any depth in the sea as a percentage of that just over the surface, the measurements being made in the same way. The values may then be ascertained readily in terms of any unit which was found convenient for the determination of the irradiation in air. This method is used in the present paper. The transmissive exponent,[3] k ("absorption coefficient"), can be readily derived from the percentage data by applying the usual formula:

$$\frac{I}{I_0} = e^{-kL}$$

where $L =$ thickness of water layer (expressed in meters) in which the intensity is reduced from I_0 to I. Convenient tables for the calculation involved are provided by Sawyer (1931).

The spectral sensitivities of our photo-electric cells (without the cases, but with the diffusing discs in position) have been determined by the U. S. Bureau of Standards. The curves are reproduced in Fig. 2, where it will be seen that no significant differences exist. For purposes of standardization the range of sensitivity of the photometers was not considered to extend to the extreme limits of the curves, for to do so would result in the inclusion of a relatively large amount of the standard lamp's radiation in the yellow which would be responsible for a very small part of the photo-electric current. The limits of the range of sensitivity of the photometers were arbitrarily set at 3460A° and 5260A° respectively. This range extends 900A° above and below the point of average maximum sensitivity (4360A°) and includes over 96 per cent of the total area of each curve.

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[3] It is the *vertical* transmissive exponent that is actually measured. The question of the direction of light rays beneath the surface has been discussed by Atkins (1932, p. 184).

The standardization of each photometer was carried out against a 1000-watt Mazda " C " lamp, manufactured, seasoned, and rated by the General Electric Company. The data supplied give the irradiation

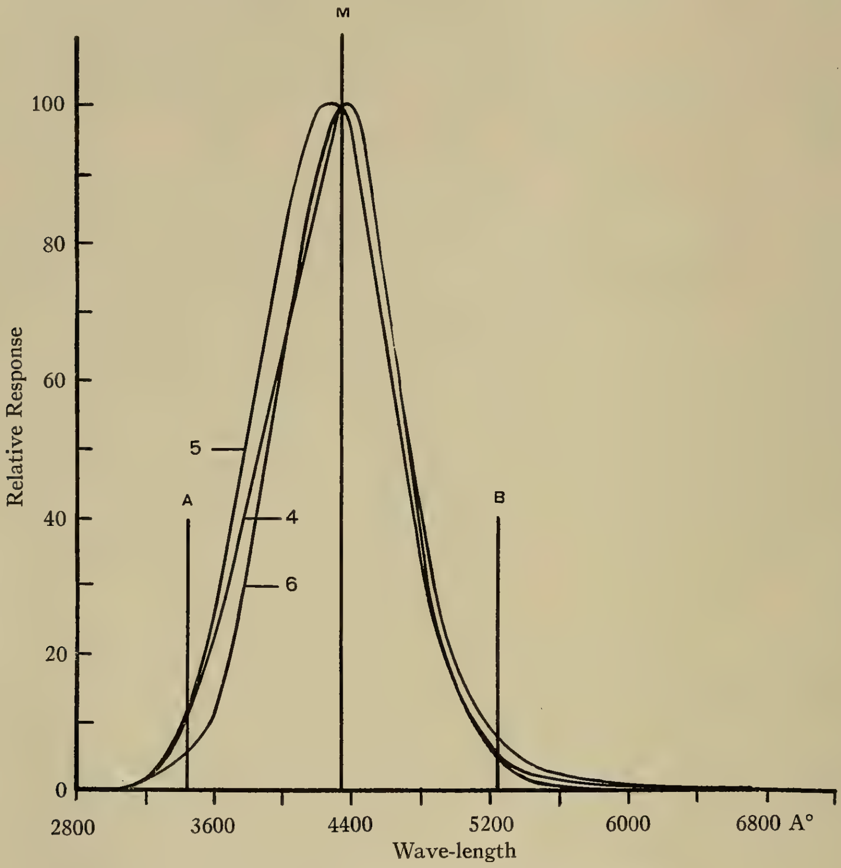

FIG. 2. Comparison of spectral sensitivities of photometers.

Curve 6: No. 7276—vacuum potassium (sensitized) cell—deck photometer.
Curve 5: No. 7275— " " " " —No. 1 sea photometer.
Curve 4: No. 7274—Gas-filled " " " —No. 2 " "

Each curve represents the relative response to an equal energy spectrum of the combined photo-electric cell and its diffusing disc of opal glass. For purposes of standardization the sensitivities are considered limited to the range between *A* and *B*, which are 900A° above and below *M*, the point of average maximum sensitivity.

$A = 3460$A°
$B = 5260$A°
$M = 4360$A°

present in a designated direction at a distance of one meter from the lamp (color temp. = 2835° K at 105.0 volts). The irradiation is expressed in microwatts/cm.[2] for each wave length interval of 100A° from 3200A° to 10,000A°. From these data the irradiation at the photometer window (placed one meter from the lamp) within the designated range of spectral sensitivity of the photometers was calculated and found to be 126 microwatts/cm.[2] By dividing this value by the photo-electric current produced, expressed in microamperes, the sensitivity of each photometer was found in terms of the irradiation necessary to produce a photo-electric current of one microampere. A summary of the standardization is given in Table I.

Changes in Emission of Photometers

Probably the greatest source of error during the measurements was the occurrence of changes in the emission of the photometers. It is unfortunate that there was no opportunity before the apparatus was assembled for testing the photo-electric cells in complete detail, as is recommended by Shelford (1930). When the "Atlantis" reached the United States and standardization was undertaken, the extent of the variability of the photometers was revealed. When the cells are irradiated in the darkroom by the standard lamp, the current emitted diminishes gradually during the course of the exposure.[4] This is particularly true of the gas-filled cell. This change of emission (formerly called "photo-electric fatigue") takes place chiefly within the first few minutes of exposure and rarely exceeds 10 per cent in magnitude (cf. Campbell and Ritchie, 1930; and Harrison, 1930).

Another more serious change in sensitivity was found to occur after an exposure of the photometer to intense irradiation (e.g. sunlight) for an hour or more. The response to the standard lamp following such treatment was greater—sometimes by as much as 30 per cent—than the response beforehand. Accordingly, when employed at sea, the deck photometer was probably less sensitive in the morning, after a night in darkness, than it was in the afternoon, after exposure to the noonday sun. Moreover, when either sea photometer had been used under water for an hour or more and then replaced on deck beside the deck photometer, it did not show the same ratio to the other instrument as before. It is hoped that in the future a satisfactory method can be devised for preventing the photo-electric current from surpassing the limit above which constancy is lost without reducing the range of intensities which can be measured.

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[4] The potential was always applied continuously to the cells for at least ten minutes before readings were begun, thus avoiding any "soakage effects" in the cable.

During the observations of 1931, no method for checking the photometers existed except by comparing them with each other. However, there is no evidence that the error due to changes of emission ever exceeded 30 per cent and probably was rarely greater than 15 per cent. The deck photometer was almost always used with its diaphragm in place (see Gall and Atkins, 1931) so that the photo-electric current was never large. Most of the submarine measurements were made with the vacuum cell photometer No. 1, which has been found to be more stable than the others. The values obtained for submarine irradiation in terms of percentages of the light falling upon the surface are considerably more accurate than the absolute measurements. Since the deck photometer and the sea photometer were compared before and after each series, corrections for any changes in sensitivity could be made.

For the work of 1932 a portable test lamp, similar to that employed by Poole and Atkins (1928), was constructed. It consists of a seasoned 50-candle-power auto headlight bulb mounted in a copper shade which fits snugly over the photometer window. The photo-electric emission produced in each photometer by this test lamp (run at 5.00 volts) is carefully observed just after standardization (cf. Table I). Assuming that any changes observed to occur in the emission produced by the test lamp represent corresponding changes in the sensitivity of the photometer to daylight, the absolute value of the irradiation measured at sea can be calculated. The constancy of the test lamp is vouchsafed by checking against the standard lamp from time to time.

Other Sources of Error

As light travels through the water, its nature becomes changed in two ways: First, its intensity is reduced; second, its spectral range is diminished by the progressive elimination of the longest and the shortest rays.[5] Any photometer with a broad spectral sensitivity would accordingly register a reduction in illumination due to a combination of these two effects, and the parts played by each would be indistinguishable. This difficulty can be removed to some extent by placing suitable light filters over the cells, or by using cells which are sensitive to only a narrow range of the spectrum. As will be seen from Fig. 2, the present method falls in the latter category. It must be borne in mind, however, that nothing can be said as to the distribution of the irradiation within the range of sensitivity. The error from this source is fortunately small due to the relatively narrow and steep shape of the sensitivity curve.

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[5] For the relative parts played by pure absorption and by scattering, see Martin (1922) and Hulburt (1932).

A more serious chance of misinterpretation results from the fact that observations on the degree of penetration of an individual color do not give an accurate measure of the change in total irradiation in the sea. Our photometers are sensitive to the group of wave lengths which is believed to penetrate the farthest in clear sea water (Grein, 1914; Hulburt, 1932; Atkins and Poole, 1933; and Sawyer, 1933).[6] Accordingly, the change in total irradiation with depth is greater than is indicated by the measurements in the present paper. Ideally, each color should be investigated separately, and then the results added together to provide quantitatively accurate values for total irradiation. Although limited regions of the spectrum are chiefly responsible for influence upon certain vital functions (e.g. photosynthesis; cf. Klugh, 1930), the whole spectrum—or a large part of it—is undoubtedly effective in many other cases.

The measurements presented in this paper are subject to a number of other possible sources of error, common to all observations of this type, the importance of which it is difficult to estimate. A brief enumeration may be made as follows:

(1) Errors due to the shape and diffusing quality of the receiving surface of the photometer, and to the occurrence of " dark currents " in the measuring circuit. This last trouble has been reduced to small proportions in our apparatus, due to the excellent quality of the insulation (Gall and Atkins, 1931).

(2) Errors due to changes in the cloudiness of the sky, in the elevation of the sun, and in the condition of the sea surface. Possibly the sea may be sufficiently smooth at times so that the light rays are not scattered at the surface to a significant extent, but under other conditions diffusion is undoubtedly so great that the surface of the sea is, in effect, the source of irradiation. Since the nature and the extent of the action of the surface under different conditions is not thoroughly known, and since in certain cases the angle of incident light has been found unimportant (Atkins, 1932, p. 184), no correction for the altitude of the sun has been attempted in this paper (cf. Birge and Juday, 1930). Whatever effects the surface is found to exert, the results will differ according to the method of measurement used, and, accordingly, great

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[6] In determinations of the spectral absorption of sea water in which parallel rays chiefly are measured (cf. Sawyer, 1931) the rays of short wave length, which are scattered more than those of long wave length, are prevented by the apparatus from re-scattering back to the photometer as they do to some extent in measurements at sea. The transmission of the longer wave lengths would therefore appear relatively greater than under conditions where the scattered light was also measured. This fact should be borne in mind when making comparisons with other investigations. Suspended matter and stains may modify color selection profoundly (Shelford, 1929; Birge and Juday, 1931, 1932).

caution must be exercised in making comparisons with other investigations. In the present undertaking the values obtained for the upper water layers were checked in as many cases as possible with the neon lamp outfit (cf. Gall and Atkins, 1931).

(3) Errors due to the shadow of the ship and errors made in the estimation of the depth of the photometer. The ship was hove to with the stern directed as nearly as possible toward the sun, but in the work of 1931 it was not practicable to lower all the sails. The expanses of white canvas undoubtedly affected the deck cell, and in 1932 all the sails were lowered during measurements. The submarine photometer was suspended over the stern from the flagpole (in 1931) or from the end of the mizzen boom (in 1932). The heave of the ship caused a vertical movement of the photometer in the water, the amplitude of which was always noted (greatest extent, 3 m.), and the average depth recorded for each observation. This depth was corrected for horizontal displacement by multiplying by the cosine of the angle which the supporting cable made with the vertical for each observation. Since the cable probably was not straight under water but curved downward to some extent, this method of calculating the depth gives a slight over-correction (cf. Poole and Atkins, 1926).

(4) Errors due to the drifting of the ship into regions of water of different transparency in cases where horizontal distribution of suspended matter is irregular, and errors due to vertical changes in transparency caused by the diurnal migration of plankton. Observations on the actual effect of the plankton are discussed below.

These four possible sources of error are adequately discussed by Shelford and Kunz (1929), Shelford (1930), Poole and Atkins (1928, 1929), and Atkins and Poole (1933). There is no reason to suppose that the errors from these causes are any greater in this investigation than in those to which reference is made, and it is believed that the precautions taken, as outlined above, have actually reduced the magnitude of certain of the errors.

Observations

The observations made while crossing the Atlantic in 1931 are summarized in Table II. The complete curves for all of the series will be found in Figs. 3 and 4. The curves represent the irradiation at each depth expressed as a percentage of the light falling upon the surface and plotted on a logarithmic scale. The slope of each curve is therefore proportional to the value of its transmissive exponent. In each case the ratio of the photometers when the sea photometer was just over the surface is taken as 100 per cent. Measurements were ordi-

TABLE II

Summary of 1931 observations

Series No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Station No.	1002	1005	1007	1010	1013	1016	1019	1023	1029	1033	1038	1041	1053
Lat. N.	52° 17′	51° 17′	50° 09′	48° 27′	47° 04′	44° 59′	43° 13′	41° 45′	38° 13′	37° 27′	36° 56′	36° 55′	43° 27′
Long. W.	29° 46′	30° 03′	29° 57′	29° 58′	29° 59′	30° 08′	30° 10′	30° 33′	31° 39′	31° 44′	44° 52′	52° 41′	67° 39′
Date 1931	July 26	July 27	July 28	July 29	July 30	July 31	Aug. 1	Aug. 2	Aug. 4	Aug. 6	Aug. 12	Aug. 15	Aug. 24
Local sun time (24-hr. scale)	1133–1233	1235–1400	1205–1330	1130–1245	1115–1205	1110–1215	1100–1300	1103–1208	1253–1353	1223–1453	1545–1745	1055–1230	1010–1120
Av. altitude of sun	57°	46°	50°	60°	58°	60°	65°	61°	55°	54°	27°	64°	49°
Photometer No.*	1	1	1 N	1 N	1 N	1 N	1 N	1	2	1 N	1, 2	2	1 N
Av. transmissive exponent, k, over depth studied	0.10	0.10	0.061	0.058	0.051	0.052	0.049	0.055	0.044	0.053	0.046	0.031	0.12
Depth at which irrad. is reduced to 1%, *meters*	34	37	71	68	84	82	86	76	97	85	91	149	32
Range of irrad. on deck during series† (3460A°–5260A°), *microwatts/cm.²*	16,000–37,000	9,000–18,000	13,000–16,000	18,000–38,000	10,000–53,000	9,000–24,000	11,000–42,000	37,000–38,000	28,000–35,000	11,000–37,000	4,000–19,000	22,000–36,000	6,000–8,000
Sea (Douglas Sea Scale)	Moderate	Moderate	Moderate	Moderate	Moderate	Rough	Slight	Smooth	Moderate	Slight	Calm	Smooth	Rough
Sky	Clear	Overcast	Overcast	Few clouds	Many clouds	Many clouds	Few clouds	Clear	Few clouds	Few clouds	Few clouds	Clear	Overcast

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* N in photometer column indicates that the measurements just under the surface and in the upper water layers were checked with the neon lamp outfit.

† Some of these values may be too high due to the probable increase in sensitivity of the photometer after use in very intense light.

narily made every ten meters. The value for each depth is the average of at least three readings. All measurements below the surface have been corrected for "external reflection" and "internal reflection" by multiplying by the factor 1.09 (cf. Atkins and Poole, 1933, p. 135).[7] The first point on the graph gives the percentage beneath the surface, at a depth (1 to 1.5 meters) just sufficient to prevent the photometer from coming out of the water when the ship pitched. The value obtained represents the reduction of light due to loss at the actual surface and to the absorption by the layer of water above the photometer.

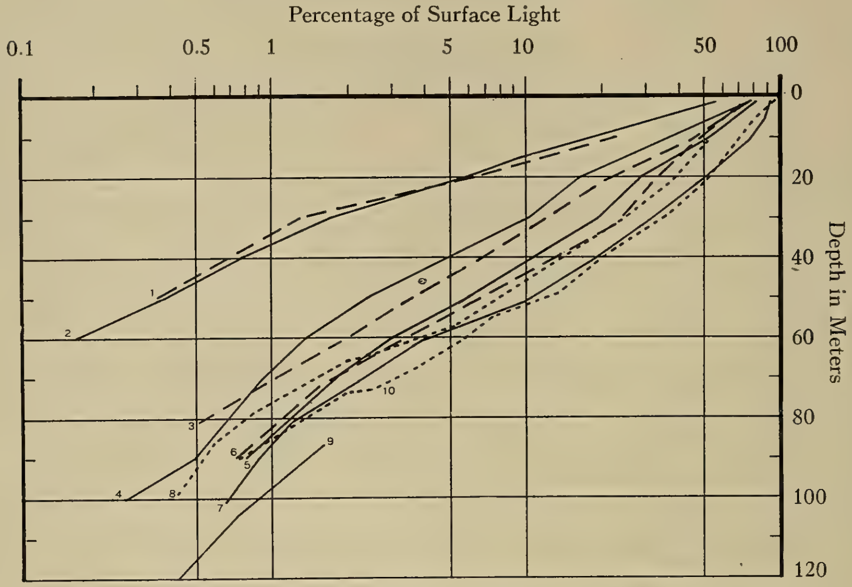

FIG. 3. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale). The series shown (1 to 10) were made in a line running south on longitude 30° W. The figure at the end of each curve designates the series number of that curve. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

The first ten series of observations were made on a line running north and south—approximately longitude 30° W. The observations were made in order from Series 1, the northernmost (lat. 52° N.), to Series 10, the southernmost (lat. 37° N.). It will be seen from Fig. 3 that the transparency of the water increases as one proceeds toward the south. The average transmissive exponent is the same for the first two series ($k = 0.10$). For Series 3 the transmissive exponent is much reduced ($k = 0.061$). The value diminishes more gradually thereafter, reaching a minimum in Series 9 ($k = 0.044$). The values obtained in Series 5, 8, and 10 do not follow the sequence.

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[7] The use of this factor has resulted in values of 100 per cent or greater for certain of the measurements just beneath the surface. The source of error in these cases is not known.

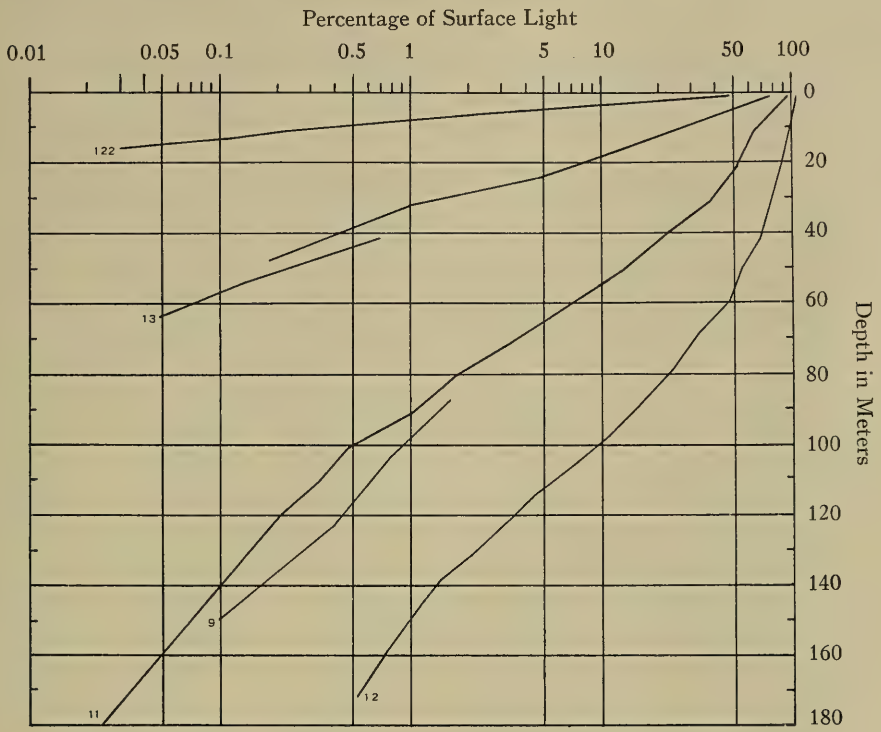

FIG. 4. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale).

Series 9—near Azore Islands.
Series 11 and 12—Sargosso Sea.
Series 13—Gulf of Maine.
Series 122—Woods Hole Harbor.

Series 12 and Series 122 represent respectively the most transparent water and the least transparent water encountered. The anomaly of the curve for Series 12 starting above 100 per cent is discussed in a footnote in the text.

The sudden reduction in the value of the transmissive exponent found between Series 2 and 3 is coincident with the sharp division between northern water and southern water as marked by the northern branch of the Gulf Stream. Toward the south the surface water is warmer and in addition the stratum of warm water extends to a much greater depth (cf. Iselin, 1934). The lesser transparency of the northern water may be due to a greater amount of suspended matter (sediment and plankton) carried here from distant regions, or to a greater growth of plankton occurring in this region as a result of more favorable physical and chemical conditions.

A closer examination of the curves in Fig. 3 reveals that the differences in the transparency of the water at these ten stations are greatest within the upper 20 meters. In the first place great variation is found in the amount of light transmitted through the surface itself. The irradiation just under the surface (1 to 1.5 meters) varies from 57 per cent to 92 per cent. The lower values for the northern series may be due in part to the rougher weather experienced at those stations (cf. Atkins, 1932, p. 191). In the second place, the transmissive exponent of the subsurface water itself varies, for it is seen that the curves diverge rapidly from 1 meter to about 20 meters. From here on they are more nearly parallel. Since the sea was not smooth at any of these stations (except one), the change in altitude of the sun with latitude probably would not account for this change in transmissive exponent (cf. Atkins, 1932, p. 184). A greater amount of suspended matter in the upper 20 m. might account for the decreased transparency in this layer at the northern stations.

The curves for Series 11 and 12, made at stations 10° and 20° further to the west, are shown in Fig. 4. To facilitate comparisons with Fig. 3, the curve for Series 9 is repeated. The horizontal stray of the instrument was responsible for the fact that no greater vertical depth than 180 m. (calculated from wire angle) could be reached although the full 200 m. of cable was used. More irregularity was observed in the measurements at these great depths than normally characterized the shallower readings. This was undoubtedly due to fluctuations in the depth of the photometer which were not accurately reflected in the angle of the supporting cable. For the lower part of the series, the curves have been drawn as an average between the points rather than from point to point as in all other cases. The increased slope of the curve of Series 12 shows that the water at this station was more transparent ($k = 0.031$) than at any other station. However, the physical and chemical properties of the water were found to be the same as at the station where Series 11 was made (Iselin, 1934). The latitude of the two stations is practically identical. Since Series 11 was made in the late afternoon, whereas Series 12 was made at noon, the difference in elevation of the sun may have been responsible for the difference in transparency in this particular case. During both these observations the sea surface was smooth and probably did not diffuse the light nearly as much as in the cases discussed above.

The curves for the southern stations (Series 6 to 12) show a rather striking similarity in shape. The slope is steep at first, then becomes more gradual, and finally assumes a steep angle again. At the more easterly stations, the first change in slope occurs between 20 and 30 meters, the second change between 60 and 80. In Series 11 and 12 these changes fall respectively between 30 and 40 meters and between 100 and 140 meters. Evidently there exists an intermediate zone of decreased transparency which is found at greater depths at stations where the water is clearer. It is possible that this zone is similarly encountered at the northern stations, but that here it exists at a higher level and extends to the surface.

Series 13 was made in the Gulf of Maine under adverse weather conditions. The transparency is very slightly less than in Series 1 and 2. The remaining curve in Fig. 4 represents Series 122 made a year later in Woods Hole Harbor. The water here was found to be the least transparent encountered, light being reduced to 1 per cent at a depth of 8 meters. The curves of Fig. 4 therefore represent the extent of the range of transparencies observed during the two years work.

In 1932 eight series of observations were made in the Gulf of Maine. These are summarized in Table III. Curves plotted for the five series at Station 1287 are shown in Fig. 5. The loss at the surface, as indicated by the measurements at one meter, varies over a considerable range. Inspection of Table III shows that there is no simple relation between this loss and any one of the meteorological conditions. The value of this measurement probably results from a complex interaction of the elevation of the sun, the condition of the sky, and the state of the sea surface upon both reflection and refraction. However, the value of the transmissive exponent, from one meter down, varies very slightly during these four days. Diverse refraction at the surface may account for some of the differences observed, and the errors of measurement are probably responsible for others. The ship may have drifted into regions where varying amounts of suspended matter existed in the water. It is important to note that the diurnal vertical migration of zoöplankton has no measurable effect upon the transparency of the water in this case. The data presented elsewhere (Clarke, 1933) indicate the numbers of animals involved and the magnitude of their movements. The level of the zone of maximum abundance changes with the hour of the day. There are no corresponding changes in the transmissive exponent at the depths involved. Wherever steeper segments of the curve do occur, they do not correspond with zones of abundant zoöplankton.

Three series of measurements were made in Woods Hole Harbor from the power boat "Asterias." The data for these are presented in

TABLE III

Summary of Gulf of Maine and Woods Hole Series

Series	13	112	113	114	115	116	117	118	119	120	121	122
Station	1053	1285	1285	1286	1287	1287	1287	1287	1287	W. H. Harbor	W. H. Harbor	W. H. Harbor
Lat. N.	43° 27′	42° 34′	42° 34′	42° 30′	43° 04′	43° 04′	43° 04′	43° 04′	43° 04′	41° 31′	41° 31′	41° 31′
Long. W.	67° 39′	69° 37′	69° 37′	69° 37′	69° 24′	69° 24′	69° 24′	69° 24′	69° 24′	70° 40′	70° 40′	70° 40′
Date	Aug. 24 1931	July 9 1932	July 9	July 10	July 10	July 11	July 11	July 11	July 12	Aug. 16	Aug. 22	Aug. 22
Local sun time (24-hr. scale)	1010–1120	1302–1352	1907–1942	0652–0732	1526–1632	0805–0847	1203–1252	1837–1907	0612–0707	1149–1429	1029–1134	1339–1429
Av. altitude of sun	49°	64°	9°	16°	41°	29°	64°	15°	13°	61°	41°	50°
Photometer No.	1 N	1	1	1	1 N	1 N	1 N	1 N	1 N	1	1	1
Av. transmissive exponent, *k*, over depth studied	0.12	0.15	0.12	0.14	0.16	0.14	0.14	0.16	0.16	0.43	0.47	0.49
Irrad. at depth of 1 meter (% surface light)	78	100 *	69	78	87	55		72	55	44	48	48
Range of irrad. on deck during series (3460A°–5260A°), *microwatts/cm.²*	6,000–8,000	16,000–17,000	200–700	5,000–7,000	6,000–11,000	7,000–13,000	15,000–16,000	600–1,300	4,000–7,000	12,000–15,000	11,600–12,400	11,800–12,400
Sea (Douglas Sea Scale)	Rough	Slight	Moderate	Moderate	Slight	Rough	Moderate	Moderate	Slight	Calm	Calm (Glassy)	Calm
Sky	Overcast	Few clouds	Few clouds	Few clouds	Overcast	Overcast	Clear	Few clouds	Clear	Clear	Clear	Clear

* The explanation for this value, which is obviously too high, is not known (see footnote in text).

Table III and the curve for Series 122 is shown in Fig. 4. In this series measurements were made every meter to a depth of 16 meters. Since the water in the harbor is so rapidly changed by swift tidal currents, considerable fluctuations in the amount of sediment present undoubtedly occur. In Series 121 it was possible to measure the irradiation with the photometer suspended so that its window was only a few centimeters below the surface. The irradiation was found to be 84 per cent of that recorded a few centimeters above the surface. Two hours later when Series 122 was made, a gentle breeze had sprung up and it was not possible to make another measurement as near the surface as before. The transmissive exponent for the whole series was slightly increased, but the irradiation at one meter remained the same.

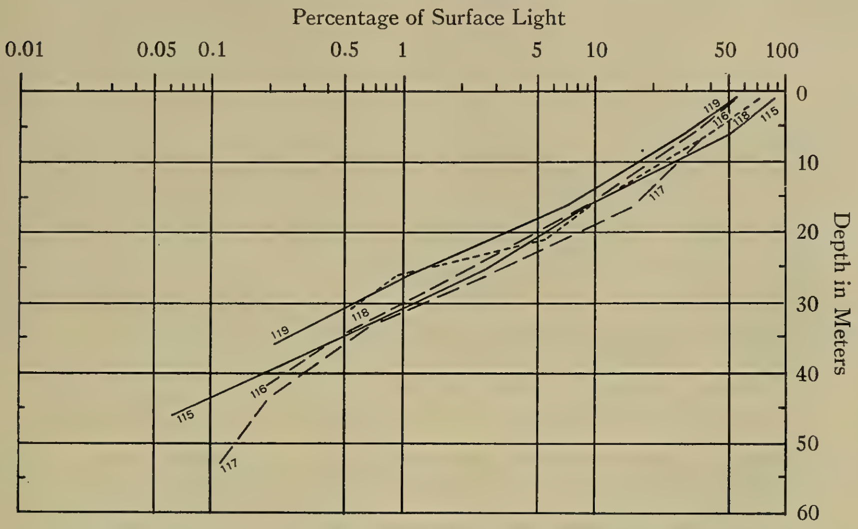

FIG. 5. Relation between depth and irradiation expressed in percentage of light just over the surface (logarithmic scale). The series shown (115 to 119) were all made at Station 1287 in the Gulf of Maine. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

DISCUSSION

A thorough review of the literature on the transmission of solar radiation through water has recently been made by Atkins (1932). Accordingly, no systematic comparison of the work of previous investigators will be attempted in the present paper. However, the general relationship between observations reported here and comparable investigations will be discussed briefly.

Comparisons can be made directly with the work of Poole and Atkins since our photometers have been standardized against theirs (see Table I). The values for surface loss vary within the same range in both investigations. The transmissive exponents found to characterize the water at Stations 1002 and 1005 and at Stations 1053 and 1287 are of the same magnitude as those observed by Poole and Atkins (1929) in the English Channel.

The measurements at stations farther to the south give transmissive exponents lower than any previously reported using the photoelectric cell. Employing photographic methods, Grein (1914) detected blue-violet light in the Mediterranean at 1500 meters; and Helland-Hansen (1931) found blue light at 500 meters in the Sargasso Sea.[8] Shelford and Gail (1922) reported blue light at 120 meters in Puget Sound amounting to about 0.2 per cent of the surface light. This indicates a transparency approximately equivalent to our Series 11. The transmissive exponent recorded for Series 12 ($k = 0.031$) approaches the value regarded by Atkins (1932, p. 185) as possible for the clearest ocean water. The values for the upper 59 meters in this series are:

meters	k
1–21	0.010
21–31	0.013
31–41	0.011
41–49	0.028
49–59	0.015

The transmissive exponent for the first 41 meters is lower than the minimum for pure water ($k = 0.015$) according to Sawyer (1931). This apparent anomaly may be due in part to the fact that Sawyer's measurement is diminished by the loss of light scattered in its passage through the water. In our measurements this loss is compensated to some extent by light which is scattered back into the cone of radiation received by the photometer. It does not seem likely that this effect could account for the whole discrepancy. Evidently, the measurements include some error. The fact that the value just under the surface is greater than 100 per cent also indicates this (see footnote above). However, one can hardly escape the conclusion that the transparency of the water at this station was exceptionally great—perhaps near the maximum possible.

The least transparent water observed—in Woods Hole Harbor—was found to have a transmissive exponent comparable to that reported by Poole and Atkins for certain measurements in the English Channel, especially those made near shore in the Plymouth region. The water of many of the Wisconsin lakes investigated by Birge and Juday shows a similar degree of turbidity. The transparency reported by Shelford (1929) for San Juan Channel is intermediate in value between that found in Woods Hole Harbor and that found in the Gulf of Maine. The present measurements, therefore, fall in line with those made by other investigators and, to a certain degree, extend our knowledge of the penetration of light into the sea.

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[8] See Atkins and Poole (1933, p. 154) for discussion of the conditions in the Sargasso Sea. Dr. Atkins informs me also that the color of the water on the Forel scale is 2 in this region as contrasted with 6 in the region where our first series was made.

SUMMARY

1. Measurements of the penetration of daylight into the sea were made in the mid-Atlantic, in the Gulf of Maine, and in Woods Hole Harbor, using the photo-electric method described previously. A four-conductor insulated cable and an electrically operated internal shutter have been added to the No. 1 sea photometer.

2. All three photometers exhibit essentially the same spectral sensitivity. For purposes of standardization the range of sensitivity is considered to extend from 3460A° to 5260A° with a maximum at 4360A°.

3. The photometers have been standardized against a 1000-watt Mazda lamp, the value of the irradiation from which was limited to that occurring within the spectral range of the photometers. The results of standardization are presented in Table I.

4. The changes in emission of the photometers and other possible sources of error are discussed, particularly those not dealt with by previous investigators.

5. The observations made approximately on longitude 30° W. reveal an increasing transparency of the water, particularly of the upper 20 meters, toward the south. Toward the north, the transparency is slightly greater than that found in the Gulf of Maine (transmissive exponent, $k=0.15$).

6. The most transparent water encountered was in the Sargasso Sea where measurements were made down to 180 meters. In Series 12 the average exponent was $k=0.031$, and values of $k=0.015$ to $k=0.010$ were obtained in the upper water layers. The least transparent water was found in Woods Hole Harbor ($k=0.49$).

7. Examination was made of the extent to which the altitude of the sun, the condition of the sea surface, and the vertical distribution of the zoöplankton affected the degree of penetration of light. Five series of observations under varying conditions at one station in the Gulf of Maine revealed no significant change in transparency.

8. The measurements of submarine irradiation are found to be commensurate with those of previous investigators, but certain significant differences are pointed out.

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REFERENCES

Atkins, W. R. G., 1932. Solar Radiation and its Transmission through Air and Water. *Jour. du Conseil,* **7**: 171.

Atkins, W. R. G., and H. H. Poole, 1933. The Photo-electric Measurement of the Penetration of Light of Various Wave-lengths into the Sea and the Physiological Bearing of the Results. *Phil. Trans. Roy. Soc. London,* Ser. B, **222**: 129.

Birge, E. A., and C. Juday, 1929–1932. Solar Radiation and Inland Lakes. First Report. *Trans. Wis. Acad. Sci. Arts and Letters,* **24**: 509. Second Report. *Ibid.,* **25**: 285. Third Report. *Ibid.,* **26**: 383. Fourth Report. *Ibid.,* **27**: 523.

Campbell, N. R., and D. Ritchie, 1930. Photo-electric Cells. Second Edition. London. Sir Isaac Pitman and Sons, Ltd.

Clarke, G. L., 1933. Diurnal Migration of Plankton in the Gulf of Maine and its Correlation with Changes in Submarine Irradiation. *Biol. Bull.,* vol. 65. In press.

Gall, D. C., and W. R. G. Atkins, 1931. Apparatus for the Photo-electric Measurement of Submarine Illumination Assembled for the U. S. A. Research Ship Atlantis. *Jour. Mar. Biol. Ass'n,* **17**: 1017.

Grein, von Klaus, 1913. Untersuchungen über die Absorption des Lichts im Seewasser. Teil I. *Ann. de l'Institut Oceanogr. de Monaco,* T. 5, Fasc. 6.

Grein, von Klaus, 1914. Untersuchungen über die Absorption des Lichts im Seewasser. Teil II. *Ann. de l'Institut Oceanogr. de Monaco,* T. 6, Fasc. 6.

Harrison, T. H., 1930. The Photo-electric Cell as a Precision Instrument in Photometry. Photo-electric Cells and their Applications. Edited by J. S. Anderson, Phys. and Opt. Soc., pp. 118–127.

Helland-Hanson, B., 1931. Physical Oceanography and Meteorology. *Rept. Michael Sars N. Atlant. Deep-sea Exp.,* 1910, **1**: 43.

Hulburt, E. O., 1932. On the Penetration of Daylight into the Sea. *Jour. Opt. Soc. Am.*, 22, No. 7, p. 408.

Iselin, C. O., 1934. Papers on Physical Oceanography and Meteorology. Woods Hole Oceanographic Institution. In press.

Klugh, A. B., 1930. Studies on the Photosynthesis of Marine Algae. No. 1. Photosynthetic rates of Enteromorpha linza, Porphyra umbilicalis and Delesseria sinuosa in red, green, and blue light. *Contr. to Can. Biol. and Fish.*, N.S., **6**: 43.

Martin, W. H., 1922. The Relation between Light Absorption and Light Scattering for Liquids. *Jour. Phys. Chem.*, **26**: 451.

Poole, H. H., and W. R. G. Atkins, 1926. On the Penetration of Light into Sea-water. *Jour. Mar. Biol. Ass'n,* **14**: 177.

Poole, H. H., and W. R. G. Atkins, 1928. Further Photo-electric Measurements of the Penetration of Light into Sea-water. *Jour. Mar. Biol. Ass'n,* **15**: 455.

Poole, H. H., and W. R. G. Atkins, 1929. Photo-electric Measurements of Submarine Illumination Throughout the Year. *Jour. Mar. Biol. Ass'n,* N.S., **16**: 297.

Russell, F. S., 1927. The Vertical Distribution of Plankton in the Sea. *Biol. Rev. and Biol. Proc. Camb. Phil. Soc.*, **2**: 213.

Russell, F. S., 1931. The Vertical Distribution of Marine Macroplankton. X. Notes on the behavior of Sagitta in the Plymouth area. *Jour. Mar. Biol. Ass'n,* **17**: 391.

Sawyer, W. R., 1931. The Spectral Absorption of Light by Pure Water and Bay of Fundy Water. *Contr. Can. Biol. and Fish.*, N.S., **7**: 75.

Sawyer, W. R., 1933. *Contr. Can. Biol. and Fish.*, N.S. In press.

Shelford, V. E., 1929. The Penetration of Light into Puget Sound Waters as Measured with Gas-filled Photo-electric Cells and Ray Filters. *Contr. from Zool. Lab. Univ. Ill.*, No. 349 and from *Puget Sound Biol. Sta.*, **7**: 151.

Shelford, V. E., 1930. Further Notes on the Acquisition and Use of Photoelectric Cells. *Ecology,* **11**: 348.

Shelford, V. E., and F. W. Gail, 1922. A Study of Light Penetration into Seawater made with the Kunz Photo-electric Cell with Particular Reference to the Distribution of Plants. *Publ. Puget Sound Biol. Sta.*, **3**: 141.

Shelford, V. E., and J. Kunz, 1929. The Use of Photo-electric Cells for Light Measurement in Ecological Work. *Ecology,* **10**: 298.

THE POLYMORPHIC FORMS OF MELITTOBIA CHALYBII ASHMEAD AND THE DETERMINING FACTORS INVOLVED IN THEIR PRODUCTION. (HYMENOPTERA: CHALCIDOIDEA, EULOPHIDÆ)

RUDOLF G. SCHMIEDER

Zoölogical Laboratory, University of Pennsylvania

Introduction

The purpose of this paper is to describe the polymorphism which occurs in *Melittobia chalybii* Ashm.,[1] as observed in rearings made in the laboratory during the winter of 1928–29, and which, so far as known, has not heretofore been recorded for any species of *Melittobia*. This polymorphism involves the interpolation in the normal life-history of a generation of individuals which are genetically like the type-form but differ from that form in their structure and in their habits. In addition to the two forms of males and of females normally occurring, a third form of female was observed rarely. This form was also obtained under certain experimental conditions.

Melittobia chalybii resembles closely the European species *M. acasta* in its structural features, its habits, and its life-history. The latter insect has been quite thoroughly studied, in this country by Howard and Fiske (1911) and in Europe by a number of investigators, most recently by Graham-Smith (1919), Browne (1922), and Picard (1923). As a result of these studies the biology of *M. acasta* is now rather well known.

There is a decided structural antigeny, the males differing from the females in the reduction of their wings, the possession of an ocellus-like pigment spot on either side of the head in place of compound eyes, and in the modification of their antennæ.

The habits of *M. acasta* are also well known. It parasitizes the larvæ of numerous species of bees, wasps, and their hymenopterous parasites as well as the puparia of parasitic diptera in the nests of wasps and bees. In the laboratory it has likewise been reared upon the larvæ of ants and the pupa of a cerambycid beetle. This polyphagous habit of the species has been made in part the basis for a stimulating discussion on the problem of host relations by Thompson and Parker (1927).

Careful studies of the life-history of *M. acasta* have been carried out, especially by Browne (1922) and Picard (1923), and the behavior of both sexes described in detail. The males are greatly outnumbered by the females, constituting only 5 per cent of the population. These males have excited the interest and the admiration of all observers by reason of their extraordinary courage and their ardor in love. They are very belligerent and engage in mortal combats with each other, not so much to gain the favors of the females but from sheer inherent pugnacity. Mating occurs within the cocoon or puparium, as the case may be, of the host and only the females eventually make their way to the outside. In the case of bees and wasps, female chalcids gain access to a host larva by entering the cells before they have been completed or they may, according to Thompson and Parker (1927), gnaw their way into the nest and into the cocoon of the host. Having found a host larva, the chalcid remains on its body, puncturing it by means of the ovipositor and in this way obtaining food and apparently also paralyzing it and preventing further development.

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[1] The species was kindly determined by Mr. A. B. Gahan.

Hundreds of eggs are deposited upon the host in the course of the parasite's long life of three months, so that in spite of the small size of the chalcid larva (less than 2 mm.), a comparatively large host larva, such as that of one of the larger wasps, is completely devoured.

Parthenogenesis occurs, but virgin females lay only a few eggs which invariably develop into males. Such male offspring, according to Browne, mate with and fertilize the mother, which then begins to oviposit freely.

Buckell (1928) has described the life-history of *M. chalybii* from observations on several generations reared in the laboratory at Vernon, British Columbia, during the winter of 1926. The biology of this species was found to correspond in many of its details rather closely to that of *M. acasta,* as briefly outlined above.

In the present paper, therefore, lesser details of the life-history and of the behavior of *M. chalybii* will not be discussed at length, except in so far as they may be necessary for the purpose of this article, or as they differ markedly from those previously recorded for this insect.

Material and Methods

The chalcids used in this investigation were obtained from the mud-nests of *Trypoxylon politus* Say found on the walls in a deserted house at Goshen, Cape May County, New Jersey, in September 1928. Additional material was obtained from the nests of *Sceliphron cæmentarius* Drury and *Chalybion cyaneum* (Fab) collected near Doylestown, Pennsylvania, during December of the same year.

The host larvæ used in the experiments were found in the cells of the above-mentioned nests and included *Trypoxylon, Chalybion,* chrysids, and an ichneumonoid parasite. In most of the work *Trypoxylon* served as the host although identical results were obtained with the use of the other species named.

All experimental rearings were carried out in small covered glass chambers or cells constructed for the purpose. It made no difference in the results when these were kept in the bright light on the laboratory table or in the dark incubator; nor is the maintenance of any degree of humidity a problem, since all larvæ transform perfectly whether they have been kept outdoors during the winter or have been allowed to lie entirely uncovered in the dry atmosphere of the heated laboratory.

The individual parasites used in starting the first series of experimental cultures were obtained from a single cocoon of *Trypoxylon* chosen from among many which were brought indoors during September. This cocoon, like many others, was fairly filled with the larvæ of *M. chalybii,* five hundred or more, all fully grown. Pupation, shortly followed by the emergence of adults, began towards the middle of November. Virgin females were readily secured by selecting and segregating pupæ of that sex, and all fertile females were obtained by mating such virgins, on the day they emerged, with individual males. In the first experimental series, there were employed ten of these females, all fertilized by a single male from the same host cocoon, and six other females which were left unmated. The mating of every fertile female used was observed under the binocular microscope. Each female was then placed in an individual culture chamber together with a host larva free from its cocoon.

Life History

An external examination of a *Trypoxylon* cocoon does not reveal whether or not it contains *Melittobia.* The material of the cocoon is hard, brittle and black, contains imbedded sand grains and is entirely intact. This indicates that the female chalcid gained entrance to the cell before the cocoon was spun and must have remained on the body of the host larva during the spinning process as suggested by Buckell and observed in *M. acasta* by Browne. On the other hand, Thompson and Parker state that *M. acasta* gnaws its way into the cocoon of its host and indicate that it also first bores its way through the mud wall of the nest.

Feeding

Soon after a female has been placed on a host larva feeding activities commence; punctures through the integument of the host are made with the ovipositor, and subsequently these punctures are revisited and the minute bits of blood which have exuded are ingested. Previous writers have stated that some of these " stings " with the ovipositor are accompanied by the injection of a paralyzant which immobilizes the host and prevents its transformation. *Trypoxylon* larvæ during the winter diapause are extremely flaccid and are entirely incapable of any movement, paralyzation being therefore superfluous. As regards interruption of development of the host, it was found that the " stings " of the chalcid were not always efficient, at least not in the case of several larvæ from *Sceliphron* cocoons in which pupation, succeeded by the hardening of the ectoskeleton, intervened and prevented the feeding and development of the parasitic chalcids.

Feeding activities continue until the end of the female's life, usually a period of from 60 to 75 days. Whether her death ensues from old age or from her inability to obtain food from the host, which by that time has been rather completely consumed by her own offspring, was not determined.

Oviposition

By the eleventh or twelfth day the abdomen of the female has become greatly distended and oviposition begins. During this process the ovipositor does not penetrate the host, the point of it is merely pressed against the surface, and the egg, flowing out between the valves of the ovipositor, comes to lie on the surface of the host. In *M. chalybii* it was found that for the first three or four days eggs are laid at the rate of four or five per day. Whether this rate increases or decreases later on is not known, but it is safe to assert that a productivity of thirty-one eggs per day, as recorded by Browne for *M. acastæ,* is not attained. *M. acasta* also differs in that it may begin oviposition within twenty-four hours after emerging.

Development

After an embryonic period lasting four days the young larvæ hatch and begin to feed on the surface of the host. The first few larvæ (from 12 to 20, when reared on *Trypoxylon*) then go through a very rapid development and emerge as imagoes within a minimum period of 14 days after oviposition. Some of them, it appears, may require a few days, perhaps as much as a week, longer. These imagoes, both males and females, are quite different from the type form of the insect in their appearance and in their behavior. The length of their imaginal life is also abbreviated, most of the females not surviving longer than three days, although some may live 15 to 20, or even 30 days. Never do these individuals attempt to leave the cocoon of the host within which they were born, but after mating deposit their eggs upon the same host upon which their mother continues to oviposit. After the above-mentioned first few larvæ have transformed, the remaining larvæ derived from the eggs laid somewhat later by the original female, together with the larvæ hatching from the eggs laid by the short-lived females of the second-form (usually over five hundred or six hundred in number), experience a much longer period of development and do not emerge until about 90 days after oviposition. In Table I these two forms of individuals are compared. The figures for the type-form represent averages from a number of rearings; also they were not obtained from the observation of individual specimens but from the records of the stages present each day in the cultures. The figures for the second-form represent the average minimum periods; the total maximum would probably exceed the minimum by not more than a week.[2]

TABLE I

Comparison of time of development and length of imaginal life of type-form and second-form of M. chalybii

Stadium	Type-form	Second-form
	days	*days*
Embryo	4	4
Larva	71	7
Pupa	15	3
Total	90	14
Longevity of the ♀	60–75	2–30

COMPARISON OF THE TWO FORMS

Imago

The outstanding structural differences between the two forms may be understood by comparing the figures on Plate I. In the males of the type-form three well-developed dorsal ocelli are present on the vertex; while in the second-form these may be entirely absent in some individuals, or in others from one to three may be present in a more or less aborted condition and without pigment. The large, facetted eyes present in all females are aborted in both forms of the male, and are represented, in the type-form male, by a black ocellus-like spot on either side of the head. In the second-form male they are similar but the pigment is again absent and only the small oval windows in the integument can be discerned. The wings in the second-form, although completely expanded, are even smaller than in the type-form. In color the second-form males are of a dark reddish-brown, compared with the lighter brown of the type, a distinction which is most pronounced on the head.

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[2] Buckell (1928), in rearing several generations of *M. chalybii* at Vernon, British Columbia, indoors, during the winter of 1926 found that the times of development for the various stages were as follows: in the male, 3, 11, and 7 days for the egg, larva, and pupa respectively; in the female 4, 15, and 7 days. Browne (1922), from his rearings of *M. acasta* gives 2–9 days for the egg, and 8–9 days for the larva "under the most favorable conditions of food and temperature" and 7 days as the shortest pupal period, adding that both larval and pupal periods vary considerably in their duration.

In comparing the dimorphic females, it will be noted that in the second-form the wings remain practically in the condition in which we find them in the pupa, being short and crumpled. The insect never moves them. The abdomen of the second-form is very much enlarged and distended at the time of emergence. A thinner ectoskeleton in which the individual sclerites are often less completely differentiated, especially on the abdomen and in the antennæ where we may encounter a partial fusion of the joints, also characterizes the second-form. These females are brown in color while those of the type-form are a pitchy brownish-black.

The structural differences between the individuals of the type-form and of the second-form are associated with equally striking differences in their physiological characteristics and in their behavior. As regards the males of the type-form, it was found that they behave and carry out their life functions in a manner closely resembling the descriptions of previous authors for this species and for *M. acasta,* but with some exceptions.

The males of this species, as of the other, apparently do not feed at all and are unfriendly to other individuals of their own sex.[3] The act of copulation has been described in detail by Parker and Thompson.

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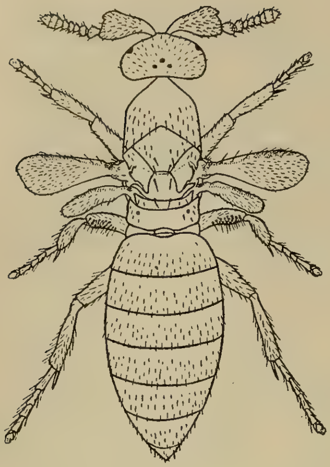
[3] The terrible duels between males, described by a number of observers for *M. acasta* and by Buckell for *M. chalybii,* did not occur often. Even when a number of males were confined together in close quarters, with or without females, their encounters were generally without serious consequences. When two males happen upon each other they both kick their legs vigorously, as an insect does when becoming entangled in a web, and as a result tumble about awkardly, often rolling over together, but then quickly separate again as each goes on his way. Only in one instance did I observe two males wrestling together for a longer time. This encounter lasted ten minutes and ended only when one individual had succeeded in chewing off the left middle-leg of his antagonist, producing a large hole in the thorax at the point of attachment. This feature of the behavior of the males is mentioned here because it differs considerably in degree from that described for the same species by Buckell.



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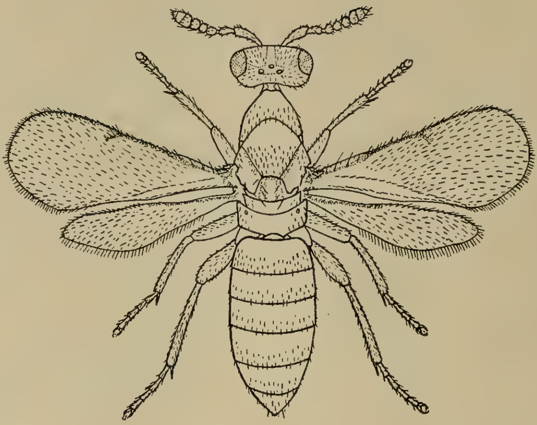

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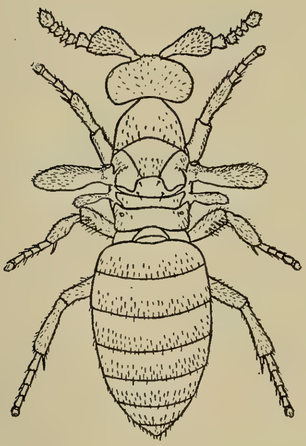

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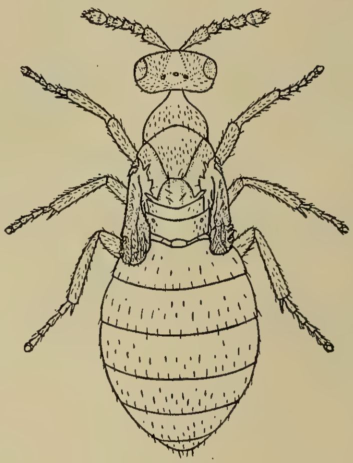

4

PLATE I

Melittobia chalybii Ashm.

FIG. 1. Type-form, male.
FIG. 2. Type-form, female.
FIG. 3. Second-form, male.
FIG. 4. Second-form, female.

In my observations, it was found to differ somewhat in the two forms. Preliminary movements go on for a period of two minutes followed by actual contact of the organs for about two seconds. The males of the second-form differ mainly in that the movements of their antennæ and legs during copulation are less regular and methodical, less perfectly coördinated, and that the entire act requires not more than one-half minute for its completion. This difference in the time given to the preliminary movements preceding insemination proved troublesome in experiments in which the males of one form were mated with females of the other form, since the coöperating final reactions of the female of each form are synchronized to the actions of the males of that form.

The males of both forms are comparatively short-lived; their constant activity and abstinence from food soon depletes their energy reserves and they die within a week after emergence.

Feeding and egg-laying activities as carried out by females of the type-form have already been described. It is these females which, after mating, make their way out of the cocoon and out of the nest of the host.[4]

The second-form of females is very different from the type-form in that the abdomen is very much enlarged at the time of pupation, the ovaries and eggs apparently developing during the pupal instar; and from 40 to 60 eggs may be laid on the day the adult emerges. The imperfections in the physical development of these females are paralleled by their very awkward and helpless behavior. They are barely able to crawl about slowly upon the host on which they were born; they mate and deposit their eggs in quick succession in the course of the first few days of their adult life. By the third or fourth day their abdomens become shrunken and collapsed as the supply of eggs is depleted and they die. Occasional females of this form are able to feed, and the peristaltic movements of their stomach are visible through the side of the abdomen. Such individuals may live and continue to oviposit for a period up to 30 days, the average being 14 days. In general, individual females are more apt to feed and continue to live if placed upon a fresh host where they are not disturbed by the presence of other adults and larvæ and where they can obtain nourishment more readily.

Larva and Pupa

Striking differences between the type-form and the second-form of individuals are equally evident in the larval and pupal stages. The shortened life-history of the second-form involves, as we have noted, an acceleration of development in the larval and pupal stages, but not in the embryo. The larva grows with great rapidity and to a larger size than does that of the type-form. In seven days it may be fully developed, after which defecation occurs followed by pupation, the pupa of the second-form being easily recognized by the large, distended abdomen. Four days after pupation, the imago appears.

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[4] Contrasting the type-form female of *M. chalybii* with the female of *M. acasta,* it is noted that in the former oviposition does not occur until the eleventh or twelfth day after feeding has begun. At the time of emergence the abdomen is rather slender, indicating that the ovaries have not yet fully developed, but after eleven days of feeding, the abdomen has become greatly distended and the insect begins to deposit eggs.

The larvæ which give rise to the type-form of the species remain as larvæ for a period of about seventy-five days [5] at room temperatures [6] during the winter.

Of this long larval period only the first week or two are given over to feeding, the actual time depending on the number of larvæ present and the condition of the host. During the remaining time the larvæ are in a kind of diapause, and it is this type of larva which hibernates in the cocoons of the host. A remarkable and puzzling circumstance is that although the host is always completely consumed by the parasite larvæ so that there remains only the shrivelled integument containing a quantity of white material, the urates of the fat body, these parasite larvæ are all perfectly developed. None of them appears undernourished and each without exception eventually transforms. Although the possibility of cannibalism among the larvæ is not entirely excluded, evidences of such behavior were not apparent. Neither form of larva spins a cocoon.

In both forms of larvæ, as in all other parasitic hymenopterous larvæ, the discharge of wastes and food residues occurs only once, just preceding pupation. In these chalcids, the egesta of the type-form consist, as previously described by Buckell, of a long dry chain of frass pellets, several times the length of the larva and gray in color. In the second-form the egested material is yellowish and semi-liquid, solidifying only after exposure to the air.

Discussion

When the life cycle as it probably occurs in nature is reviewed, its adaptive nature becomes immediately apparent. A female *Melittobia* of the type-form may enter the cell of a *Trypoxylon* before that cell has been sealed shut and remain there while the host larva is developing. During this time the chalcid may feed upon the larva or even upon the spiders with which the nest has been provisioned, and when the wasp larva spins its cocoon the chalcid becomes permanently enclosed within it and may then begin depositing its eggs. The parasite is very minute compared with its host and lays but four or five eggs per day, too low a rate to furnish sufficient offspring to consume the host entirely, especially if oviposition is begun late in the summer.[7] However, fourteen days after the first eggs have been laid, a generation of offspring consisting on the average of 13 females and 2 males of the short-lived or second-form of individual emerges. These remain within the cocoon and the females after mating each deposit from 40 to 60 or more eggs upon the same host. It is these eggs, together with the eggs the original mother continues to lay on this host, which give rise to the 500 to 800 larvæ finally produced, a number sufficient to use up entirely the available food. All of these larvæ, some the offspring of the original female but the majority the offspring of her daughters of the second-form, follow the longer course of development and after a diapause finally transform into imagoes of the type-form. It is larvæ of this kind which one finds hibernating in the cocoons of the host.

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[5] This time is the average of 20 cultures in which the date when the maximum number of eggs were present in each culture was noted and compared to the date on which the largest number of larvæ were pupating.

[6] The temperatures in the laboratory during the winter varied somewhat from day to day, ranging from 19° to 25° C., the usual temperature being 23° C., the room remaining at this temperature both day and night.

This interpolation of an additional generation of adults in the life-history is thus seen to constitute a remarkable biological adaptation which effects a more complete utilization of each host and, as a corollary, secures the production of a maximum number of offspring from each host. The structural characteristics of the two forms and their behavior also reflect the difference in the rôles they fulfill in the survival of the species. The type-form is produced in large numbers and is adapted to making its way out of the host cocoon, leading an active, " outdoor " life and seeking new hosts upon which to oviposit. While this form assures the dispersal of the species, the second-form is adapted only to life within the host cocoon chosen by its mother and its function is purely a reproductive one. Although the means used to secure the end are different, the final outcome of the production of this second-form of individual may be compared to the results of polyembryony in the life history of certain other chalcid flies.

Factors Conditioning the Production of the Polymorphic Forms

Life Cycle

Under natural conditions, it is only the first few eggs laid by a type-form female which develop into individuals of the second-form; the other eggs as well as all of the eggs of the second-form females always develop into the type-form.

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[7] From the length of the life history it appears that there are probably two generations of the type-form each year, one which emerges in the spring from the overwintering larvæ, and a second which emerges in the late summer.

The questions which naturally arise are: Is there a difference in the eggs themselves which causes the first few to develop along a different direction from that followed by the eggs laid somewhat later, or are there other factors operating? Again, can individuals of the second-form produce only the type-form of insect, is there any regular alternation of forms involved, and are the two forms in any way genetically different?

In the ordinary course of the life cycle, the type-form female lays some eggs which yield second-form adults and others which yield again the type-form. If we let "T" represent the type female and "S_1" the second-form, then the succession of forms occurring in nature may be represented as follows:

$$\begin{tikzcd}
& S_1 \arrow[r] & T \\
T \arrow[ur] \arrow[rr] & & T
\end{tikzcd}$$

However, if a female of the second-form is removed from the host upon which it was born and would ordinarily remain to deposit its eggs, and is placed upon a fresh host which has not yet been parasitized, we get the same end results as if we were using a female of the type-form. In other words, the first few eggs of this second-form female produce a generation of individuals like herself; the subsequent eggs, type-form individuals. Diagrammatically, we may represent the succession thus:

$$\begin{tikzcd}
& S_2 \arrow[r] & T \\
S_1 \arrow[ur] \arrow[rr] & & T
\end{tikzcd}$$

If we again remove the second-form imagoes to a fresh host we obtain the same results as before and this procedure can be continued, apparently indefinitely, so that we may secure a succession of generations of the second-form without the insertion of a generation of the type-form. This was actually carried through in the experiments, to the seventh generation. In the diagram each individual is shown giving rise, as it normally does, to type-form individuals also, as in the preceding description.

$$\begin{tikzcd}
& S_1 \arrow[r] \arrow[dr] & S_2 \arrow[r] \arrow[dr] & S_3 \arrow[r] \arrow[dr] & S_4 \arrow[r] \arrow[dr] & S_5 \arrow[r] \arrow[dr] & S_6 \arrow[r] \arrow[dr] & S_7 \arrow[dr] & \\
T \arrow[ur] \arrow[dr] & & T & T & T & T & T & & T \\
& T & & & & & & &
\end{tikzcd}$$

Crossing the Dimorphic Forms

Crosses were made in order to determine whether any genetic factors were involved in the production of the two forms and whether the males from eggs of virgin females [8] were in any way different from those produced by fertilized females. The various matings made are indicated in Table II, using the same notation as before. Of the crosses listed only the first and the fifth occur in nature in this species. " (V) " means that the male in question was the offspring of a virgin female.

TABLE II

Matings Made in Crossing Dimorphic Forms of M. chalybii

♀ ♂
1. T × T
2. T × S_1
3. T × S_1(V)
4. S_1 × T
5. S_1 × S_1
6. S_2 × S_1
7. S_2 × S_1(V)
8. S_2 × S_2

♀ ♂
9. S_2 × S_2(V)
10. S_2 × S_3
11. S_3 × S_2
12. S_4 × T
13. S_4 × S_3
14. S_5 × S_3
15. S_6 × S_5

None of these rearings gave results which differed at all from those already described. They rule out any possible genetic mechanism as involved in this dimorphism, and rearings number 2, 4, and 12, which are among those that do not ordinarily occur, demonstrate that a female of either form mated with either kind of male may produce offspring of both forms.

Trophic Conditions

The fact that always only the first few larva which feed upon a particular host develop into individuals of the second-form while all others which may come after are destined to be of the type-form, suggested that possibly the food which the first larvæ obtain is qualitatively different from that available to the later ones. It is found to be invariably the case that after from twelve to twenty second-form larvæ have been reared on any *Trypoxylon* larva, it is no longer possible to rear anything but type-form larvæ upon that host. This fact was established by allowing females which had not yet oviposited to lay eggs on hosts from which a generation of second-form adults had already emerged. In such cultures, even the first eggs which the female lays yield only type-form adults. When eggs laid by a type-form female, after she has already produced the full number of second-form adults, were transferred to a fresh host, they too developed into offspring of the second-form, although if they had been allowed to remain on the original host they would have yielded only type-form individuals. Experiments in transferring larger numbers of eggs to a host entirely out of contact with adult chalcids rule out the possibility that a substance injected into the host by the ovipositing female may in some way affect the feeding larvæ.[9]

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[8] Virgin females feed upon the host larva as do the fertilized females and their abdomens become equally distended. However, very few eggs are actually deposited, (from 0 to 10 in number). Of these the majority never hatch, but shrivel and dry up in the course of a few days. Occasionally, an egg will hatch and eventually develop into a male. It seems as if the proportion of eggs which hatch and produce males compared with the number which fail to develop is about the same as the ratio of males to females normally occurring in this species. From this follows the possible theoretical explanation that there is a predetermination in the ovary, that approximately 10 per cent of the eggs may develop parthenogenetically into males, while the remaining eggs cannot develop at all unless fertilized, when they produce females.

Other observations confirm the conclusion that there is a difference in the nature of the food taken up by the larvæ and that the food ingested by the first larvæ may be described as being richer. These observations are: (1) The larvæ of the second-form (those that feed first) grow more rapidly and attain a larger size. (2) Their excrement, as already indicated, is small in quantity and of a different color and consistency from that of the type-form, which is quite large in quantity and probably composed mostly of urates derived from the host. (3) When a smaller larva, *e.g.*, that of a chrysid, is the host, a correspondingly smaller number of second-form larvæ develop.

The conclusions then are, that those larvæ which are the first to feed upon a given host ingest the blood, while those which begin feeding somewhat later ingest to a larger extent the remaining available tissues, and that this trophic difference determines whether they will become adults of the second-form or of the type-form.

The Time and Nature of the Determination

In an attempt to gain some insight into the nature of the determining process and the time when it occurs, a few preliminary experiments were conducted. These consisted of transferring larvæ which had attained from one-third to one-half their full length from one host to another.

A. Larvæ from a fresh host were transferred to rather completely spent hosts on which several hundred larvæ had already fed. These larvæ, had they been allowed to remain on the first host, would have developed within 9 days into adults of the second-form.

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[9] Another but very remote possibility, namely that the larvæ which feed first may themselves inject substances into the host which affect the larvæ which feed after them, was not investigated. It would seem, however, that the larvæ producing such a secretion would themselves again ingest it.

Of 4 larvæ which were transferred, 1 completed its development on the second host in 29 days, yielding a type-form female, the other 3 died. In another experiment, again using 4 larvæ, the same result was obtained, except that the surviving individual required 30 days to complete its development. The death of most of the larvæ is ascribed to the difficulty they had in securing food from the used-up hosts and to the fact that decay had begun to set in in these used hosts.

B. Larvæ from a partly spent host were transferred to fresh, unused hosts. These larvæ, if left on the original host, would have required about 90 days to complete development and would have emerged as individuals of the type-form.

In one experiment, 4 larvæ after being transferred, all completed development within 12 days and emerged as females of an intermediate form, with wings somewhat shorter than in the type-form. In another trial, the time required was 13 days, for 2 of the 4 larvæ used. In a third, using 3 larvæ, 2 emerged on the twentieth day. All imagoes obtained were of the intermediate-form. The records for the last two experiments are incomplete due to interruption of the work.

Although the size and age of the larvæ were not carefully controlled and although the experiments were few in number, the records obtained do give some information regarding the dependence of the length of larval life and of the form of the adult upon the nature of the food taken in during different parts of the feeding period.

In the first series it is apparent that the shortening of the developmental period associated with feeding upon a fresh host is not lost when the larvæ are transferred to a used host, although the poorer food obtained from this second host does retard development by about 20 days. Either the trophic conditions during the latter part of the feeding period or the lengthening of the time of development, for the two are inseparable here, resulted in the production of individuals of the type-form.

In the second series, transferring larvæ to a fresh host accelerated their development so that they emerged, in most cases, as soon as if they had begun their feeding upon that host. But in spite of this rapid development and larger final size, characteristic for second-form individuals, the imagoes actually obtained resembled more closely those of the type-form. They are here designated as of an intermediate-form. The wings, although only two-thirds normal length, are fully expanded, as in the type-form; the abdomen is somewhat distended at the time of emergence but is not as large as that of the second-form, while in color they are again almost as dark as the type.

From these experiments it has become apparent that the length of larval life is directly determined by the trophic conditions obtaining, especially those obtaining during the latter half of the feeding period.

It appears probable (from Series B) that the production of the type-form is determined during the earlier larval period, but that this tendency will not be entirely realized if the entire larval period is shortened by transferring the larva to a fresh host. In this event, it appears as if the wings did not have time to grow to their full normal length during the very brief pupal stage, intermediate-forms resulting.

The development of second-form adults seems to depend both upon the early trophic conditions and upon the shortening of the larval and pupal periods. More definite or detailed conclusions cannot be ventured until more extensive experiments have been made.

CONCLUSION

The polymorphic forms in *M. chalybii* have been found to differ from each other both morphologically and physiologically and have been shown to be conditioned by extrinsic trophic factors. Both in the existence of structural differences between the forms and in the nature of the factors which produce them, the polymorphism in this chalcid is therefore comparable to that occurring in the social bees and wasps.

In *Melittobia,* as in the social forms, the food is not to be considered as a direct cause of the differences between the polymorphic forms, but rather as a factor which acts in an indirect way upon the growing larva and determines which one of two inherently possible paths of development will be followed. The restriction of the insect to one of two alternatives in development is especially obvious in species like *Melittobia chalybii* and the honeybee, in which intermediates between the two forms of the female are exceedingly rare, in spite of the fact that, at least in the former species, all trophic gradations from the most to the least favorable are experienced by numerous individual larvæ. In bumblebees, on the contrary, we find less difference between the two forms of the female, and the intermediate forms seem to indicate a more direct dependence upon the amount or the quality of the food received by the larva.

Finally, it is of interest to recall here that in one of the few other instances of polymorphism in chalcids, that of *Trichogràmma cacœciæ,* an egg parasite, Marchal has found that the larvæ are also sensitive to food differences and react by a change in the rate of development, this rate depending upon the rate of development of the host embryo.

Summary

1. The parasitic chalcid *Melittobia chalybii* Ashm. occurs in two forms, the type-form and the short-lived or second-form. The production of one or the other of these two forms is determined by the trophic conditions obtaining during the growth of the larva. A third form of female was obtainable experimentally and very rarely in the normal cultures.

2. The males of the second-form differ from the type-form in their darker color and in the more aborted condition of their optic organs. The females of the second-form differ from typical females in their lighter color, in the failure of their wings to spread upon emerging from the pupa, in the large swollen condition of the abdomen which is full of ripe eggs at the time of emergence, and in the shortness of their imaginal life. Both sexes of the second-form emerge as adults in a minimum of fourteen days after oviposition compared with the ninety days required for the development of individuals of the type-form.

3. The life history is as follows: A female of the type-form enters the nest of a host wasp (*e.g., Trypoxylon politus* Say), and remains upon the host larva within the cocoon, feeding and ovipositing for about 70 days, until it dies. The first few (12 to 20) eggs laid develop in about 14 days into a generation of males and females of the second-form. These do not leave the host cocoon and the females deposit their eggs upon the same host upon which their mother continues to oviposit. All eggs, those of the mother and those of her offspring of the second-form, now develop in about 90 days into chalcids of the type-form. After mating, the females alone gnaw their way to the exterior.

4. Experiments involving the transfer of eggs from one host to another and the transfer of ovipositing females to different individual host larvæ demonstrated that all eggs of both forms of females are potentially alike and capable of developing into imagoes of either form. In every experiment, the first few larvæ which feed upon a given host invariably give rise to adults of the second-form, all the remaining larvæ to adults of the type-form. The conclusion is that the first larvæ ingest mainly the blood of the host while the later ones must feed to a larger extent upon the remaining tissues; and that this trophic difference determines the production of one or the other form of adult from a single type of egg.

5. Experiments involving the changing of the trophic conditions during the life of an individual larva give some insight into the nature of the determination and the time at which it occurs.

6. When larvæ were transferred from a partly consumed host to a

fresh host, a third form of female individual, termed intermediate-form, was obtained.

7. In crossing females of one form to males of the other form, including males derived from virgin females, the results obtained differed in no way from those obtained from the usual matings which occur in nature.

LITERATURE CITED[10]

BROWNE, F. BALFOUR, 1922. On the Life-History of Melittobia acasta Walker; a Chalcid Parasite of Bees and Wasps. *Parasitol.,* **14**: 349.

BUCKELL, E. R., 1928. Notes on the Life-History and Habits of Melittobia chalybii Ash. *Pan-Pacific Entom.,* **5**: 14.

GRAHAM-SMITH, G. S., 1919. Further Observations on the Habits and Parasites of Common Flies. *Parasitol.,* **11**: 347.

HOWARD, L. O., AND W. F. FISKE, 1911. The Introduction of Parasites of the Gipsy and Browntail Moths. *U. S. Dept. Agr. Bur. Entom. Bull.,* **91**: 209.

MARCHAL, P., 1927. Contribution à l'étude génotypique et phénotypique des Trichogrammes. *Compt. Rend. Acad. Sci. Paris,* **185**: 489.

PICARD, F., 1923. Recherches biologiques et anatomiques sur Melittobia acasta Walk. *Bull. biol. Fr. et Belg.,* **57**: 469.

THOMPSON, W. R., AND H. L. PARKER, 1927. The Problem of Host Relations with Special Reference to Entomophagous Parasites. *Parasitol.,* **19**: 1.

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[10] For a bibliography on *Melittobia,* consult the papers by Browne, 1922, and Picard, 1923.

PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS

PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

June 30, 1933

EFFECTS OF CENTRIFUGAL FORCE ON THE ECTOPLASMIC LAYER, NUCLEI, AND PROTOPLASM OF FERTILIZED SEA URCHIN EGGS. Ethel B. Harvey.—The ectoplasmic layer of *Paracentrotus lividus, Parechinus microtuberculatus* and *Sphærechinus granularis* can be thrown down by centrifugal force as a ring which lies in the perivitelline space. When eggs kept in Ca-free sea water are centrifuged, the ring is not thrown down, but when returned to sea water, the ectoplasmic material is precipitated as small refringent granules in the perivitelline space.

If centrifuged before or during the monaster stage, the pronuclei can be separated, the female going to the light pole and the male to the heavy pole. In greatly elongated eggs, during the time that the male nucleus is approaching the female, it may enlarge so as to become three times the volume of the female. In eggs separated into two parts, one may contain the female and the other the male nucleus. In most cases, the female nucleus forms a monaster, and the fragment does not divide; the male nucleus forms an amphiaster and this fragment divides normally. In a few cases, the part with the female nucleus divided normally and the part with the male nucleus did not divide, the male nucleus grew smaller and disappeared.

Fertilized eggs (30–90 seconds after insemination) stratify and break into parts similar to unfertilized eggs, but slightly more elongate. During the monaster stage they form long streamers sometimes 14 times the original diameter of the egg. They elongate less during the streak stage and only slightly during the amphiaster stage. The fertilized eggs of *Parechinus* and *Paracentrotus* break more rapidly at all stages than the unfertilized, the fertilized eggs of *Sphærechinus* less rapidly than the unfertilized.

MOTION PICTURES TAKEN THROUGH THE CENTRIFUGE-MICROSCOPE ILLUSTRATING DIFFERENCES IN BEHAVIOR OF UNFERTILIZED AND FERTILIZED SEA URCHIN EGGS. E. Newton Harvey.

SOME DATA FROM A CORRELATED ANATOMICAL, PHYSIOLOGICAL AND BEHAVIORISTIC STUDY OF THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG. William C. Young. (Published in the *Am. Jour. Physiol.,* **105**: 393–398, 1933.)

HÆMOGLOBIN-RINGER, A NEW MAMMALIAN PERFUSION FLUID. William R. Amberson.

July 5, 1933

SOME EFFECTS OF BLUE-GREEN ALGÆ ON LAKE FISH. G. W. Prescott.

DISTRIBUTION AND ECOLOGY OF THE MARINE ALGÆ OF THE MARITIME PROVINCES OF CANADA. Hugh P. Bell.

DISTRIBUTION OF THE FRESHWATER ALGÆ OF NEWFOUNDLAND. W. R. Taylor. —On the basis of three sets of samples examined by the writer, and a short list published by another investigator, about 500 desmids and 175 other algæ are recorded. Examination of the data for the desmids showed that they followed, in general, the distribution known to be characteristic of the Phanerogams. The special occurrences of non-desmid algæ are discussed. The extended paper is to carry in addition to this information, detailed measurements, critical taxonomic notes (including over 30 new species, varieties, and forms), and illustrations of approximately all the desmids.

July 10, 1933

THE CONCENTRATION OF ORGANIC DERIVATIVES IN SEA WATER, IN RELATION TO THE CHEMICAL COMPOSITION OF PLANKTON. A. C. Redfield.

DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION. George L. Clarke.

THE RÔLE OF BACTERIA IN THE FORMATION OF NITRATE IN THE SEA. Selman A. Waksman and Cornelia L. Carey.

July 14, 1933

THE EFFECT OF RESPIRATORY POISONS AND METHYLENE BLUE ON CLEAVAGE OF CERTAIN EGGS. M. M. Brooks.—CO (about 97 per cent) hindered cleavage of fertilized *Arbacia* and *Asterias* eggs as compared with N_2; an 80–20 mixture of CO and O_2 had little effect. KCN (5×10^{-5} M) inhibited cleavage (*Arbacia*). Methylene blue 5×10^{-5} per cent accelerated and 5×10^{-4} per cent retarded cleavage in *Arbacia; Asterias* behaved likewise but at lower concentrations. Methylene blue, 5×10^{-5} per cent antagonized the effects of CO and dilute KCN on cleavage, but not that of N_2, *i.e.,* lack of O.

IONIC CHANGES DURING THE DEVELOPMENT OF FISH EGGS. Laurence Irving and Jeanne F. Manery.

THE TENSION AT THE SURFACE OF EGG CELLS. E. Newton Harvey. (To be published in the *Jour. Cell. and Comp. Physiol.,* October, 1933.)

FERTILIZATION MEMBRANES OF CENTRIFUGED ASTERIAS EGGS. Donald P. Costello.

THE KIDNEY TUBULES WITH PHENOL RED AND NEUTRAL RED. Robert Chambers.

July 18, 1933

CHROMATIN EXTRUSION IN CERTAIN CILIATE COMMENSALS OF MUSSELS. G. W. Kidder.

THE REORGANIZATION BANDS IN THE MACRONUCLEUS OF ASPIDISCA. F. M. Summers.—The reorganization bands (Yocum, 1918) in *Aspidisca lynceus* Müll. are somewhat similar to those found in *Euplotes* (Griffin, 1910; Yocum, 1918; Turner, 1930), so that the same terminology has been adopted as far as possible.

The first indication of approaching division is the appearance of the reorganization band, which soon divides into two parts. These two bands, each consisting of three distinct planes, make their appearance (?) in the central portion of the C-shaped macronucleus and each traverses its respective half of the nucleus from the midregion to the extremity, where it disappears leaving the nucleus in a greatly altered condition.

The growth of the central region and the simultaneous disappearance of the older segments are apparently correlated with the migration of the reorganization bands through the macronucleus. Structural modifications coincident with reorganization are easily observed since both altered and unaltered parts of the nucleus are present for a time in the same organism. In Feulgen preparations the chromatin reticulum of the altered part is heavier, more compact, and with fewer alveoli than that of the unaltered parts. Flemming-hæmatoxylin preparations demonstrate certain "pocket" granules in the distal regions that are not to be found in the reconstructed part.

Following Turner's (1930) interpretation of the phenomenon in *Euplotes patella* it is suggested that the three planes of each band represent three successive phases in the transformation of the central chromatin reticulum into non-staining nuclear substances. The non-staining phase (the reconstruction plane) may be a region of interplay between altered and unaltered parts of the nucleus and between these and the cytoplasm.

COMPARATIVE STUDIES ON THE OSMIOPHILIC AND NEUTRAL-RED-STAINABLE INCLUSIONS OF THE GENUS VORTICELLA. Harold E. Finley.—Osmic acid and silver nitrate techniques were used to prepare whole mounts and sections of *Vorticella convallaria* (Linnæus 1758), *V. microstoma* Ehrenberg 1830, and *V. campanula* Ehrenberg 1831. Neutral red and Janus green B were used as vital stains. Observations indicate that very few significant differences exist between these species of *Vorticella,* so far as osmiophilic and argentophile and neutral-red-stainable inclusions are concerned. Some possible sources of errors in the identification of the "so-called Golgi material" in the Protozoa are pointed out.

EXPERIMENTAL CYTOLOGY OF AMŒBA PROTEUS. S. O. Mast and W. L. Doyle. —Amœba proteus contains among other structures: fat globules; small bodies, usually elongated; truncated bipyramidal crystals, frequently with blebs attached; and highly refractive globular bodies held by different investigators to be secondary nuclei, nutritive spheres, Golgi bodies, and vacuome.

We found the following: The small bodies have all the essential characteristics of mitochondria. The crystals are probably a magnesium salt of a mono- or disubstituted glycine. There are two kinds of refractive bodies, one in the food vacuoles, the other free in the cytoplasm. The former is optically homogeneous. The latter consists of a surface layer of viscous substance on a fragile shell of carbohydrate material which surrounds a central plastic mass.

In attempting to trace the transformation of food into protoplasm, the components of the cytoplasm were stratified by centrifuging and held in this condition by chilling, then the various structures mentioned above were partially or entirely cut out, after which the specimens were either starved or fed with selected food. The following was found:

The crystals and the homogeneous refractive bodies form in the food vacuoles. The food vacuoles divide, forming small vacuoles each containing a crystal. The mitochondria pass from the surface of the food vacuoles to the crystal vacuoles. As this occurs the homogeneous refractive bodies and the crystals become smaller and the blebs on the crystals larger until the crystals disappear and the blebs become free refractive bodies. The mitochondria appear to transfer substance from the food vacuole to the crystal vacuole. The free refractive bodies have staining characteristics of plastids in plants. They are reserve food. Both types of refractive bodies are stages in the transformation of food into protoplasm.

July 25, 1933

ONE STEP IN THE DEVELOPMENT OF HEREDITARY PIGMENTATION IN THE FISH ORYZIAS LATIPES. H. B. Goodrich and C. B. Crampton.

TRANSLOCATIONS IN THE MOUSE AND THEIR EFFECT ON DEVELOPMENT. George D. Snell.

A SERIES OF PROBABLE MUTATIONS IN DROSOPHILA PSEUDO-OBSCURA AS COMPARED WITH D. MELANOGASTER. D. E. Lancefield.

SEX-DETERMINATION IN HYMENOPTERA. P. W. Whiting.—It has been shown in mosaic males of *Habrobracon* that the dominant eye color may appear at the margin separating the genetically different tissues, each of which bears a different recessive factor. Thus black is reconstituted along the line separating ivory from white. Similarly feminized genitalia may occur if the two types of tissue adjoin in the genital region. Thus each of the two types of tissue in the male mosaic are recessive for a different factor determining sex. On the basis of these and other findings already published, it is postulated that females are in chromosome constitution, $1\ X + 1\ Y + 2\ A$, while normal (haploid) males are $1\ X + 1\ A$ or $1\ Y + 1\ A$, and biparental (diploid) males are $2\ X + 2\ A$ or $2\ Y + 2\ A$. X contains the factors F and g while Y contains f and G. The theory brings the problem of sex determination in Hymenoptera into line with current views of genic balance.

August 1, 1933

THE EFFECTS OF FAT SOLVENTS UPON THE FIXATION OF MITOCHONDRIA. Conway Zirkle.

STUDIES ON THE CYTOLOGY OF AMPHIBIA. Arthur W. Pollister.—A study has been made of the cytoplasmic structures in a variety of amphibian tissues, nearly all types being represented with the exception of the striated muscle fiber and the neurone. The Kull, Kolatchev, and Benda methods were chiefly employed. Every type of cell was found typically to contain three types of cytoplasmic structures: chondriosomes, usually in the shape of unbranched filaments; Golgi material, in the form of thin lamellæ; and, in nearly every type, a pair of centrioles. In the cells that are not physiologically polarized the Golgi platework always envelops the central apparatus that surrounds the centrioles. In cases where the cell contains an abundance of cytoplasm an aster is developed about the central apparatus, and chondrioconts in that part of the cell are oriented radial to the centrioles. To this unpolarized type belong the following cells: leucocytes, erythrocytes, mesenchyme cells, fibroblasts, gonial cells, peritoneal epithelial cells, endothelial cells, and smooth muscle fibers. In all physiologically polarized cells, *i.e.* columnar, cuboidal, and polyhedral epithelia, by contrast, the Golgi material has no fixed topographical relationship to the centrioles. The latter are usually located near the center of the distal end of the cell. The axis passing through the two is often perpendicular to the cell surface, and in some types a flagellum is attached to the distal centriole. The Golgi apparatus in most cases is in the form of a thin irregular belt, often surrounding the distal end of the nucleus. In some instances it is in the form of separate discoid lamellæ in the same position as the other type. In glandular cells the Golgi material is localized at the site of synthesis of the secretory product. In all the polarized cells the chondrioconts are oriented with their long axes parallel to the direction of the flow of materials through the cell, *i.e.* between the base and the lumen. It is suggested that cytoplasmic currents are responsible both for this arrangement and for the radial orientation of chondriosomes in the unpolarized cells.

THE IRRADIATION OF BIOLOGICAL SUSPENSIONS BY MONOCHROMATIC LIGHT. (THE EFFECT OF ULTRA-VIOLET LIGHT ON A PLANT VIRUS AND BACTERIA.) B. M. Duggar and Alexander Hollaender.

MOTION PICTURES SHOWING SOME VARIETIES OF NERVE IRRITATION, AS SEEN IN LIVING FROG TADPOLES. Carl C. Speidel.—The reactions of single internodal segments of myelinated nerve fibers in living frog tadpoles have been observed in minute detail following many varieties of experimental irritation of both acute and chronic types. Illustrative ciné-photomicrographic records have been obtained. Below are specified the titles of some of the pictures:

1. Acute irritation and rapid metamorphosis of a myelin sheath segment immediately after a hot water burn. (A continuous picture showing myelin swelling, rippling, and ovoid formation.)

2. Swelling and wrinkling of the myelin sheath following administration of a strong anæsthetic (chloretone).
3. The production of beaded myelin by alcohol intoxication.
4. The swelling reaction shown by the same two myelin segments photographed immediately before and immediately after alcohol treatment.
5. Nerve irritation from low temperature (tadpole embedded in packed snow for several hours).
6. Examples showing trophic (Wallerian) degeneration of a myelinated fiber after section.
7. Irritation and recovery of a myelinated fiber following adjacent wound infliction.
8. Traumatic irritation and recovery of a proximal stump myelin segment after nerve section.
9. Examples of myelin segments showing delayed irritation, several days after exposure to X-rays.
10. Chronic irritation resulting from starvation. (Several examples of wrinkled and swollen nerves after six days total starvation.)
11. Granular degeneration of nerve fibers and sheath cells associated with prolonged inanition (20 days starvation).
12. Effects on nerves of administration of thyroid gland extract.
13. Pressure irritation showing delicate connecting strands between the axis cylinder and myelin sheath as these two structures undergo separation.
14. Temporary irritation and recovery of a myelin segment between two wounds, the nerve fiber remaining intact.
15. Polariscopic pictures showing the behavior of the anisotropic material of the myelin sheath during various stages of growth, irritation, and degeneration.
16. Degeneration of sheath cells on normal nerves.
17. Leucocytes, chiefly macrophages, moving about in a degenerating mixed nerve.
18. A macrophage leaving a blood capillary sprout (diapedesis).
19. A macrophage containing ingested anisotropic granules moving about within a single degenerating myelin segment. (Pictures taken with both ordinary light and polarized light.)

August 8, 1933

Regional Differences in the Organization Center of the Amphibian Embryo. Edmund K. Hall. (Published in *Arch. f. entw. mech. d. organism.*, **127**: 573.)

Observations on Migrating Cells in Cultures of Amphibian Tissues, Particularly with Reference to the Problem of Fiber Formation. George A. Baitsell.

Thp Effect of Wing Bud Extirpations in Chick Embryos on the Development of the Nervous System. V. Hamburger.

Color Changes in the Dogfish. G. H. Parker.

The Absorption of Colloidal Carbon by the Mesonephric Epithelium of Necturus. A. B. Dawson.

Morphological and Electrophoretic Effects of the Galvanic Current on Griffithsia Cells. Victor Schechter.—The morphological polarity of *Griffithsia* is established very early. After many nuclear divisions the cell divides once to form the shoot, and again to form the rhizoid. By continued rapid division branching chains of cells are formed. The basal cells are attached by means of rhizoids.

For the purpose of the experiments fragments of a thallus were placed in the experimental apparatus and oriented alternately with and against the current.

After two or three days of electrical treatment a very interesting picture could be seen.

When the fragment was oriented with base toward the anode, the rhizoids originated at the basal ends of the cells—that is, toward the anode, and the appearance of the completed thallus is more or less normal. However, if the fragment was oriented with the apex toward the anode, the rhizoids were again produced toward this pole and thus at the apical ends of the cells as shown. We have here a morphological indication of reversal of polarity.

Accompanying the gross morphological effect just described are correlated intracellular phenomena of unusual interest. First, there can usually be seen in the thallus as a whole a gradient of color ranging from a deep pink in the cells toward the cathode, more intense than the usual hue of the cells, to a pale tan in the cells toward the anode. A second intracellular effect is the aggregation of chromatophores and probably other cytoplasmic components toward the anode of each cell. It may be of significance in this connection that the first indication of rhizoid production under normal conditions is the formation of a visible pigment concentration, which then pushes out as the cap protoplasm of the rhizoid.

With regard to the color changes observed, it must be of some significance that rhizoid formation is inhibited in the region which, by virtue of its position, assumes the pale tan color. A pH change may be involved. This is supported by the fact that in acid the cell pigment approximates the deep pink color observed toward the cathode of the electric field, whereas in alkali it becomes pale and finally greenish in hue. Other evidence indicates that the electrical effect on color is not due to the production of acid or alkali in the cells but rather to a migration of materials which themselves may produce relative acidity or alkalinity by derived differences in concentration. With high currents each cell soon becomes deep pink toward the cathode and green toward the anode. If the current direction is now reversed, after a few minutes the colors reverse their position. Observations during the progress of this change show a fading of the pink at the anodal end *before* any pink color appears at the cathode. In this way there is a slow shift of pink intensity from anode toward cathode, pointing to the probability that the effect is not a pH change at the cell membrane.

The possible integration of these morphological and intracellular effects must be delayed pending further data. I wish only to point out at this time the coexistence of the described intracellular effects with the determinative action of the electric current during regeneration.

ELECTROKINETIC STUDIES OF MARINE OVA. I. ARBACIA PUNCTULATA. K. Dan. *Jour. Cell. Comp. Physiol.*, Vol. 3, No. 4. 1933.

ELECTRICAL ACTIVITY OF THE BRAIN. R. W. Gerard.

August 15, 1933

THE ISOLATION OF A CRYSTALLINE GLOBULIN FROM THE ALBUMIN FRACTION OF COW'S MILK. A. H. Palmer.

STUDIES IN IRON METABOLISM IN HUMANS. Paul Reznikoff.

THE HORMONAL CONNECTION BETWEEN THE PITUITARY AND THE THYROID. Marie Krogh.

ANTERIOR PITUITARY-LIKE HORMONE EFFECTS. F. E. Chidester.

August 22, 1933

HETEROCHROMATIC RADIATION AND EARLY AMPHIBIAN DEVELOPMENT. R. Rugh.—This report deals with radiation by light within the limits of the solar spectrum of the gametes and early developmental stages of the frog.

Distinction is made between luminous and energy intensities with the suggestion that luminous flux is evaluated with reference to visual sensation and is limited to the total visible spectrum. It is not comparable to the objective physical entity of light energy measured as heat units. The two light factors controlled were light energy and wave length.

Frog gametes and fertilized eggs without jelly show the same limits of tolerance of direct sunlight. Since the pre-cleavage stages with jelly show much greater immunity to radiations than those without jelly, it is suggested that amphibian jelly may not concentrate the solar radiations but act as an insulator against them. Since jelly is 78 per cent water, it is to be expected that it would absorb heat rays.

An attempt was made to discover why monochromatic green has so often been reported as a lethal radiation, particularly in the work of Yung on tadpoles. A study of Yung's procedure indicated that neither monochromatic light nor equal energy radiations were involved. With these factors controlled, the quality of light within the limits of the visible spectrum did not matter so long as the total energy was equalized. It was suggested that green may have been lethal in Yung's work partly because he used plant food for the tadpoles and green plants cannot absorb green light, hence tadpole mortality was secondary to plant mortality.

A suggestion is made that radiation work distinguish between such factors as ultra-violet and total radiation from an ultra-violet lamp; light energy and the limited factor of luminous intensity.

A NON-LINEAR RELATION BETWEEN BIOLOGICAL EFFECT AND IONIZING POWER OF ALPHA RAYS. R. E. Zirkle.

EFFECTS OF X-RAYS UPON CELL OXIDATIONS. Leon C. Chesley.

A RESPONSE OF ARBACIA EGGS TO X-RAYS. P. S. Henshaw and D. S. Francis. —*Arbacia punctulata* eggs when exposed to X-rays before fertilization are delayed in cleavage—that is, the interval between fertilization and the onset of the first cleavage is definitely prolonged. If the amount of radiation administered is varied, the amount of effect (cleavage delay) is found to vary in the same direction, indicating that the effect is a quantitative one. Further, if time is allowed to intervene between treatment and the measurement of effect (*i.e.,* between treatment and fertilization), the effect is found to be less than when measured at once after treatment; in other words, recovery in some form or other takes place. By plotting the amount of effect against the time allowed for recovery, a die-away type of curve is obtained. This shows that the rate of recovery is rapid at first but becomes less as time for recovery increases. When various degrees of effect are produced and recovery considered in each case, a family of recovery curves is obtained which seem to bear a relationship to each other. By placing the experimental points for such a set of data on a semi-logarithmic scale, parallel straight line curves are found to fit the points as well as any that can be drawn. This indicates that the rate of recovery is the same irrespective of the magnitude of effect produced and that the recovery process at least resembles a reaction which depends on the concentration of some substance—a mono-molecular reaction. Still further consideration shows that recovery is in progress even during treatment. From this it appears that X-rays cause a slowing in the rate of cell division in case of the first cleavage in *Arbacia* eggs and that recovery from the effect begins as soon as any effect is produced.

August 29, 1933

THE COPEPOD PLANKTON OF THE LAST CRUISE OF THE NON-MAGNETIC SHIP "CARNEGIE." Charles B. Wilson.

THE PRESSURE COEFFICIENT OF VISCOSITY IN THE EGGS OF ARBACIA. Dugald E. S. Brown.

OBSERVATIONS ON LACTIC ACID, TOTAL CO_2, AND pH OF VENOUS BLOOD DURING RECOVERY FROM SEVERE EXERCISE. Edwin P. Laug.

EYE COLORS IN THE PARASITIC WASP HABROBRACON AND THEIR BEHAVIOR IN MOSAICS AND IN MULTIPLE RECESSIVES. Anna R. Whiting.—Four independently segregating loci have been identified by recessive eye color mutations which are necessarily lighter than the black wild-type. The locus orange contains light-ocelli, dahlia, orange and ivory in order of decreasing pigmentation and dominance. White, in a second locus, is partially dominant to carrot. Cantaloup and maroon are independent.

In males (from binucleate eggs of heterozygous mothers) mosaic for eye colors in the orange series there is no sharp line separating the genetically different regions. The recessive region shows more or less of the dominant character at the margin, due apparently to diffusion of a chemical substance caused by the dominant allelomorph. In contrast, eye mosaics involving the white and cantaloup loci show a clearcut line between genetically different tissues and each region is obviously autonomous. No mosaics for maroon have been found. Of special interest is the eye, mosaic for factors in two loci, such as cantaloup and ivory. The cantaloup non-ivory region is clearly marked off from the non-cantaloup ivory region, while the latter shows black at the margin grading through orange to ivory. Thus the double dominant black, comparable to the eye color of the heterozygous mother, is physiologically reconstituted although there is no genetically black tissue present. If eyes are genetically ivory but other tissue (gonads by breeding test) is black, the eyes may be uniformly orange. Thus tissues of insects are not necessarily autonomous as has been frequently assumed.

Of the forty-eight combinations of eye color factors possible in haploid males, twenty-six have been made. Of these only nine are colored. It is probable that all other combinations would prove to be colorless. Intensity of color in a single mutant type appears to have little or no relation to its effect when in combination. Double recessives are lighter than the lighter single recessive.

THE MELANIN REACTION IN RACES OF DROSOPHILA MELANOGASTER. Marc A. Graubard.

August 31, 1933

HEMATOLOGICAL STUDIES IN DOGFISH (MUSTELUS CANIS). I. THE EFFECT OF TURPENTINE ON THE BLOOD PICTURE. Paul Reznikoff and Dorothy G. Reznikoff.—The average normal blood count of 25 dogfish per cu. mm. of blood was as follows:

Hemoglobin (Sahli) 33 per cent; range (21–44)
Red blood cells (Toissins) 393,000; range (210,000–630,000)
White blood cells (Toissins) 97,000; range (60,000–178,000)
Differential (supravital stain)
  Small lymphocytes 54 per cent; range (35–71)
  Large lymphocytes (granulocytes) 9 per cent; range (2–17)
  Thrombocytes 18 per cent; range (6–32)
  Eosinophiles 14 per cent; range (5–32)
  Pseudoeosinophiles 4 per cent; range (1–8)
  Monocytes 1 per cent; range (0–2)

Frequent removal of blood from the heart (0.5 to 0.75 cc.) may or may not cause an anemia and a reduction in white blood cells depending, probably, upon the amount taken. The differential count remains unchanged. Injection of sterile water intraperitoneally has no effect upon the blood picture.

Turpentine (0.25 to 1.5 cc.) injected intraperitoneally may or may not cause some anemia but invariably causes a leukopenia with an increase of lymphocytes and a decrease of thrombocytes and eosinophiles. Just before death the thrombocytes may again rise. The following chart illustrates an example:

Time of blood examination	Hemoglobin	R.B.C.	W.B.C.	Small lymph	Large lymph	Thrombocytes	Eosin	Pseudo-eosin	Monocytes
	per cent								
Before injection	39	359,000	127,000	37	9	20	32	1	1
1 cc. turpentine injected intraperitoneally									
12 hrs.	35	393,000	70,000	79	3	13	4	1	0
24 hrs.	25	308,000	71,000	71	9	12	5	3	0
36 hrs.	36	432,000	75,000	80	4	11	4	1	0
Fish dead 40 hours after injection									

An exudate of phagocytic lymphocytes and bacteria in the peritoneal cavity in the injected as well as the control fish is found after death.

These experiments indicate that the lymphocyte is the actively responding cell to acute irritation in the dogfish and has the same significance as the neutrophile (polymorphonuclear) in mammals.

Intracranial Pigmentation in Teleosts. W. H. F. Addison.

The Blood Capillary in Relation to Contractility. E. R. Clark and Eleanor Linton Clark.—Federighi (1921), working under G. H. Parker, found in an invertebrate, *Nereis virens*, that the endothelial cells of certain blood-vessels possess a high degree of contractility; a peristaltic contraction which propels the contained blood.

Studies by the authors (1925) have shown that the endothelial cells of blood capillaries in the transparent tails of larvæ of several species of amphibians have a contractility which is decidedly less than that described for *Nereis*, but which is still a real activity which plays a part in controlling the circulation in the tail fins. No definite muscle cells are present on the arterioles or capillaries in this region, but extraendothelial cells are found which have been included by Vimtrup (1922) among cells which he calls "Rouget" cells, but which we have found non-contractile.

The morphology and behavior of blood capillaries in the rabbit's ear have been studied in our laboratory for the past seven years, by the use of double-walled transparent chambers, in which the arterioles, capillaries, and venules, including the cells making up their walls, may be seen with the greatest clearness with the highest microscopic lenses. Both the original capillaries of the ear and newly formed ones have been investigated, and it has been found by Sandison (1932) and by the authors in observations which have continued to date that the endothelial cells here are devoid of contractility, as are also the numerous "Rouget" cells. Local control of the circulation apparently resides exclusively in the arteries, arterioles, arterio-venous anastomoses and some of the larger veins, all of which are supplied with smooth muscle cells; their activity in turn depending upon an intact nerve supply.

These three sets of observations on invertebrate, amphibian, and mammal suggest an evolutionary loss of endothelial contractility associated with the elaboration of the neuro-muscular apparatus.

STRIATED MUSCLES OF THE LAMELLIBRANCH MOLLUSC, PECTEN GIBBUS. Herbert L. Eastlick.—A cytological study of the anterior portion of the adductor of *Pecten gibbus* shows that this muscle is composed of definitely striated myofibrillæ, the fibrillæ varying in size from barely discernible strands up to fibers 6 mμ in diameter. The segmented structure of the muscle is very apparent in both living and in fixed and stained preparations. A myofibril may be analyzed into the following components which seem to be homologous with similar structures in vertebrate or other highly differentiated muscle: the (Q) disc or the anisotropic segment, which is highly refractive and becomes intensely stained with hæmatoxylin; alternating with this disc is the (J) or the isotropic segment which is not very refractive and never stainable with hæmatoxylin. In the center of the (Q) disc there occurs a light area, the (H) disc or the disc of Hensen. The (J) disc is bisected by the (Z) line or membrane which is often granular and irregular in outline. In late stages of contraction and in complete contraction, never in the relaxed state, one can discern that the (H) disc is bisected by an exceedingly narrow line, the (M) membrane.

The striated portion of the adductor of *Pecten* differs from ordinary segmented muscle in that the fibrillæ are not organized into definite fibers, a true sarcolemma being absent and its place taken by a ramification of connective tissue which divides the muscle into irregularly sized bundles. Furthermore, the (Z) membrane is not continuous transversely across a muscle bundle but is limited to each myofibril; hence there is no definite transverse orientation of the discs and membranes as is always the case in vertebrate muscle.

Nuclei lie scattered at the inner surface of the connective tissue envelope surrounding a muscle bundle and in the interstices of the fibrillæ. The nuclei are spindle-shaped and in fixed preparations have an average size of 3 × 5 mμ. In the immediate area of a nucleus a clear, non-granular area of sarcoplasm occurs.

THE CENTRIOLES OF AMPHIBIAN TISSUES. Arthur W. Pollister.

THE CHROMIDIUM IN ARCELLA VULGARIS (EHRENBERG). Theodore G. Adams. —The investigation of the chromidium was made using the Feulgen reaction, Bauer's modification of the Feulgen reaction, and the mitochondrial technique of Benda.

In *Arcella* the Feulgen technique after the usual fixatives shows numerous granules of a deep purple color in the net. The network itself does not give the reaction. Methylene blue, Delafields' and Heidenhein's hematoxylin stain only the network leaving granules as clear discrete bodies. In a recent paper of Hans Bauer (1932), it is shown that chrome fixatives give better results with the Feulgen reaction that is a deeper color than when the aqueous solution of sublimate was used in Feulgen's original paper.

Bauer (1933), using the Schiff reagent, devised a test for polysaccharides. It consisted of chroming the tissue and applying the Schiff reagent without any hydrolysis at 60° C. He obtained reactions in the tissues of *Lumbricus* sp., *Helix pomatia,* and *Allium cepa* respectively for glycogen, galactogen, starch tunicin, and fructose. Inulin, a polysaccharide, did not give the reaction so that it is to be considered a presumptive test for a polysaccharide. In *Arcella* in the same clone the granules in the net gave a deep purple reaction to the foregoing test. No other structure in the cell gave the reaction. When the chroming process is supplemented by osmic acid without fat solvents in large quantities, fats and fatty substances such as Golgi material and mitochondria are preserved. These, according to Bauer (1933), react to the polysaccharide test when fixed in the forementioned manner.

In *Arcella* after the Benda fixation, bodies in the cytoplasm larger than the chromidial granules in the net react to the Bauer test for polysaccharides, with the exception that they are reddish, not purple. Some are paired unequal in size, some are equal, some are single. Some lie close to the net and have the appearance of secondary nuclei, some adhere to the nucleus, a phenomenon characteristic of the surface tension effects of fats.

Animals from the same culture, previously examined for large granules in the cytoplasm, were fixed in a 4 per cent aqueous chromic acid and 20 per cent acetic and treated with Bauer's technique. The granules in the net gave a purple reaction but there was no trace of any bead-like or paired granules outside the net.

The living cells were stained vitally with Janus green B (Farbwerk Hoechst). It was difficult to see the characteristic color as differing from the original refractive index of the granules in the cytoplasm, outside the net.

The Benda technique stained these paired granules and other large granules in the cytoplasm outside the net a purplish red. This indicates the mitochondrial nature of the granules.

It may be concluded that the Bauer test is a presumptive test of a polysaccharide for the granules in the net of *Arcella* when fat solvents have removed fatty substances from the cell.

SELECTIVE FERTILIZATION IN STYELA. Harold H. Plough.—Morgan found that removal of the chorionic membrane with the test cells from the egg of *Ciona* made self-fertilization possible. He therefore concluded that there was no incompatibility between eggs and sperm of the same animal. This conclusion might have been expected to hold for other tunicates showing self-sterility, but such is not the case.

In *Styela* (*Cynthia*) *partita* not all animals are self-sterile, but the common result of a series of reciprocal crosses is a small number of selfed eggs and a large number of crossed eggs showing fertilization and development. Since *Styela* eggs can be secured in large numbers only by allowing isolated animals to shed normally, all eggs are exposed to their own sperm before crosses are made. It turns out that with adequate controls the resultant exposure to a mixed sperm suspension in crosses is favorable for deciding the question of incompatibility of gametes.

During the past three summers Miss Evangeline Alderman and I have been studying this situation in *Styela*. It has been found that decrease or increase of the hydrogen ion concentration of the sea water, varying the salt content, introduction of *Styela* body fluid, and other changes in the normal optimum sea water only reduce fertility all along the line without any influence on self- or cross-fertility. Allowing the eggs to stand in sea water usually results in increased self-fertility and decreased cross fertility, until at the end of 3 or 4 hours approximate equality is reached.

Attempts to remove the chorionic membranes of *Styela* eggs by use of capillary pipettes have been uniformly unsuccessful. However, this result has been accomplished in about 20 per cent of cases by the use of crab digestive juice as suggested recently by Berrill (1932). There is always a general toxic effect when this fluid is used to digest off the membranes, but in the best cases many eggs develop. Strangely enough, removal or weakening of the membranes has no effect on the relation of self- and cross-fertility. The membranes appear to have nothing to do with the block to self-fertilization in *Styela*.

Finally, examination of many stained sections of eggs fixed about three hours after the final sperm exposure gives a perfectly clear picture, since the sperms are relatively large. The selfed eggs have many sperm inside the membranes and test cells and in close contact with the egg cortex. Fertilization has not taken place, as shown by the presence of the intact first maturation metaphase. In the crosses, however, the sperm do penetrate, and the eggs go into *cleavage*. Thus self-sterility in *Styela* seems to be a true case of selective fertilization.

RELATION OF TEMPERATURE AND CLEAVAGE IN FROG'S EGGS. Meyer Atlas.

DISORIENTATIONS OF DEVELOPMENT IN CREPIDULA, CAUSED BY COLD. E. G. Conklin.

CHANGES IN THE ARBACIA EGG IMMEDIATELY FOLLOWING FERTILIZATION, AS DETERMINED BY CENTRIFUGAL FORCE. Ethel Browne Harvey.—*Arbacia* eggs centrifuged 25–90 seconds after fertilization become more elongate and break into halves and quarters more readily (most batches) than unfertilized eggs. With very high speed (11,000 × gravity), they can be pulled apart before the elevation of the fertilization membrane, and the membrane may form subsequently over the parts, but is thinner over the centripetal poles. If centrifuged 1½–5 minutes after fertilization, they break still more readily and into many small pieces. In some batches, the fertilized eggs break more readily at every stage after fertilization than the unfertilized. In other batches, they break more readily only till a certain stage which varies with the different batches. By observation of unfertilized and fertilized eggs at the same time while rotating in the double-head centrifuge microscope devised by E. N. Harvey, it can be definitely determined that the fertilized eggs at all stages stratify less readily than the unfertilized and are therefore more viscous. The difference is slight in the early stages and becomes more marked later on. The greater ease of breaking apart of the just fertilized egg must be connected with surface changes rather than with changes in viscosity.

RECOVERY FROM X-RAY EFFECTS IN ARBACIA EGGS BEFORE FERTILIZATION AND ITS EFFECT ON DEVELOPMENT. P. S. Henshaw and Dorothy S. Francis.—At a previous meeting we have described a response of *Arbacia punctulata* eggs to X-rays. The results presented showed that the period between fertilization and the onset of the first cleavage is definitely prolonged if the eggs are exposed before fertilization; and further, that recovery from this effect takes place if time is allowed to intervene between treatment and measurement of the effect (*i.e.* between the end of treatment and the moment of insemination). For this consideration, recovery was measured at once after the interval allowed for this process because cleavage time begins at the time of fertilization. More recently we have determined the degree of development attained 48 hours after fertilization, in an attempt to find whether the recovery which took place before fertilization was in evidence at a later period. The results obtained showed that it is and in much the same way as when measured by the effect on cleavage time. The organisms which had had the least time for recovery were retarded the most in development and those which had had the most time for recovery were most advanced. A curve showing the rate of recovery, as measured by effects on development, resembled the one which was obtained by measuring the effects on cleavage time. Because of this similarity, it appears that whatever X-ray effect is present at the time of fertilization is fixed by fertilization and that no further recovery takes place—that is, as far as this particular irradiation effect is concerned.

ON THE RELATIVE SENSITIVITY OF ARBACIA EGGS AND SPERM TO VARIOUS ACIDS AND BASES. Anna K. Keltch, Lucille Wade, and G. H. A. Clowes.—At last year's meeting it was reported that *Arbacia* sperm appeared to be relatively resistant to butyric acid and sensitive to ammonia, whilst *Arbacia* eggs were relatively resistant to ammonia and sensitive to butyric acid. These experiments have been extended during this season to include a series of mineral and organic acids and bases and a series of alcohols. Exposures were made in isotonic saline, using concentrations of 1,000, 4,000 and 16,000 eggs per cc., and .004, .016 and .064 cc. concentrated sperm per cc. for periods of 1¼, 2½, 5, and 10 minutes.

The extent of survival was determined by returning eggs and sperm to sea water and fertilizing previously exposed eggs with normal sperm and using previously exposed sperm for fertilization of normal eggs. The results previously reported with butyric acid and ammonia were confirmed. Hydrochloric acid was found to differ strikingly from butyric acid, being highly toxic for sperm as compared with eggs.

A rough index of relative toxicity, obtained by dividing the concentration required to exert a given toxic effect on the sperm by that required to exert a given toxic effect on the eggs, puts hydrochloric, caustic soda, ammonia, and the ethyl amines in a range of 0.2 to 0.1 or less; that is, they are from five to ten times as toxic for sperm as for eggs. Organic acids of the type of citric, tartaric, and oxalic fall in a range of 0.3 to 0.5, as does formic acid, but the subsequent members of the fatty acid series, acetic, propionic and valeric, also benzoic acid, fall in the same category as butyric with an index of 2 or more. Apparently weak organic acids possessed of a great capacity for cell penetration are relatively less toxic for sperm than for eggs.

Preliminary tests conducted with ethyl, amyl, and octyl alcohols give them an index in the range of 0.3 to 0.5, thus apparently excluding surface tension as an important factor in the relative toxicity to sperm and eggs.

VARIATIONS IN THE SENSITIVITY OF EGGS FOLLOWING FERTILIZATION. G. H. A. Clowes, Anna K. Keltch, and Lucille Wade.—Using the procedure outlined in previous paper, sensitivity tests have been conducted on *Arbacia* eggs at five-minute intervals from fertilization to the third division. The curves have been compared with Heilbrunn's viscosity curves and Fry's description of egg development. As reported at last year's meeting, butyric acid and ammonia, which penetrate the egg easily, exert effects after fertilization to the point of fusion of egg and sperm nucleus differing strikingly from the effects which they exert on the unfertilized egg but corresponding approximately with that exerted on sperm, the egg during this period being relatively sensitive to ammonia and resistant to butyric acid.

As development proceeds eggs become relatively less sensitive to ammonia and more sensitive to butyric. There are points of relative maximum sensitivity to ammonia which appear to fall in the lower zone of the viscosity curves and well-defined points of maximum sensitivity to butyric acid at points of maximum viscosity, viz., that following fusion of egg and sperm nucleus and those at which 50 per cent of the eggs have divided.

Oxalic acid differs strikingly from butyric acid, showing points of maximum sensitivity in the lower zone of the viscosity curves following the breakdown of the nuclear membrane, and resistance at the peaks of viscosity, suggesting the possibility that the calcium ion may play a rôle in connection with coagulative changes associated with spindle formation and cell division.

Hypotonic NaCl, like butyric acid, exerts a maximum destructive effect at high viscosity points. The addition of calcium may eliminate the hypotonic effect at the high viscosity point following fusion of egg and sperm nucleus, and may reduce but not eliminate the point of maximum sensitivity when 50 per cent eggs have divided.

The strikingly cyclic rhythms obtained with butyric acid and the somewhat less striking but divergent rhythms obtained with ammonia, which harmonize roughly with Heilbrunn's viscosity curves and also with the periods of low intensity of staining of chromatin in the intact nucleus and high intensity of staining of the chromosomes during division, suggest that some type of acid basic cyclic rhythm plays a significant rôle in the mechanism of cell division.

THE FLATTENING OF MARINE EGGS UNDER THE INFLUENCE OF GRAVITY. E. Newton Harvey.—If egg cells are considered drops of a non-miscible liquid in water, resting on a plane surface, they should flatten, provided the tension (T) at their surface is sufficiently low or the force [gravity × difference in density between egg and medium, $g\ (d-d')$] is sufficiently great, or their radius (r) is sufficiently large. Plasticity or elastic structure would act against flattening. Within limits the flattening, f, varies as $r \times \sqrt{g\ (d-d')}/\sqrt{T}$.

Previous experiments indicate that the tension at the surface of the cells is low—less than one dyne per cm. From Heydweiller's tables and equation, it can be calculated that an egg whose tension at the surface is 0.2 dyne/cm. should not flatten 10 per cent unless its radius is over 400 μ. Since most marine eggs are smaller than this, we should not expect them to flatten appreciably unless T is very low. Observation from the side shows that "naked" eggs like *Arbacia, Asterias, Echinarachnius, Ilyanassa, Crepidula plana, fornicata* or *convexa,* and *Chætopterus* are either perfectly spherical, or, if irregular, remain so, without flattening. *Busycon* eggs (1 mm. diameter) are of the consistency of putty and do flatten sufficiently to indicate a tension at the surface of less than 0.5 dyne/cm. Eggs with a definite chorion at the surface may be spherical (*Phascolosoma*), slightly flattened when shed (*Nereis*), or disc-shaped when shed (*Arenicola*). Such eggs merely fall on their flat side and the flattening has nothing to do with the action of gravity.

THE ACTION OF ANÆSTHETICS ON THE SURFACE PRECIPITATION REACTION. L. V. Heilbrunn.

THE EFFECT OF CERTAIN SALT SOLUTIONS ON THE PERMEABILITY OF THE ARBACIA EGG. Dorothy R. Stewart and M. H. Jacobs.—It is generally believed that the absence of bivalent cations such as $Ca^{\cdot\cdot}$ from the medium surrounding a cell may cause an increased permeability of the latter. Accurate quantitative studies of this effect have been made in the case of water by Lucké and McCutcheon, but are almost lacking in the case of solutes. A method developed by one of the authors permits the simultaneous determination of "permeability constants" for water and for a penetrating solute. This method has been applied to the egg of *Arbacia,* using ethylene glycol as the penetrating solute, and comparing permeability in sea water, in isotonic solutions of NaCl, KCl, and $CaCl_2$, and in solutions containing various combinations of NaCl and $CaCl_2$. The results on the permeability of the unfertilized egg to water are in satisfactory agreement with those which Lucké and McCutcheon obtained by an entirely different method. They indicate a considerably greater permeability in NaCl and in KCl than in sea water and in $CaCl_2$. Unlike the permeability of the cell to water, that to ethylene glycol is almost the same in sea water and in the various salt solutions. The addition of $CaCl_2$ in increasing amounts to NaCl causes a progressive decrease in permeability to water while leaving that to ethylene glycol practically unaltered. In striking contrast with the effects of changing the electrolytes in the external medium are those of fertilization, which causes a great increase in permeability not only to water but to ethylene glycol as well. The fact that one type of treatment of the *Arbacia* egg increases its permeability to both substances and another type of treatment increases its permeability to water alone is of interest in connection with certain theories as to the nature of cell permeability.

THE EFFECTIVE PERIOD IN DEVELOPMENT OF THE MUTANT FACTOR "EYELESS" IN HABROBRACON. B. R. Speicher.—Eyeless wasps show malformed head capsules characterized by production of a pair of large lobes on the sides of the head which bear minute compound eyes at their tips.

In wild-type wasps the dorsal part of the head capsule is formed by four imaginal buds, an anterior pair, the antenna-cephalic discs, forming the antennæ and some hypodermis at their bases, and a posterior pair, the oculo-cephalic discs, forming the head capsule around and including the compound eyes and ocelli. These imaginal buds develop by invagination and separation from the larval hypodermis and proliferate within the larval head.

In eyeless, the antenna-cephalic discs and the anterior and ventral margins of the oculo-cephalic discs develop normally. During the late larval stage the dorsal and posterior margins of the oculo-cephalic discs fail to separate from the larval hypodermis and, being thus restricted in development, grow outward to form the lobes characteristic of the mutant type. Later development is normal except for some modification by previous abnormality. The compound eyes are not close to the optic lobes, and there are no nerve connections between the two organs.

VARIEGATED EYE COLOR IN HABROBRACON. Anna R. Whiting.—When the semi-dominant factor shot-veins is combined with the factor for white eye color (whwh.svsv), all the white eyes show red spots in the posterior ventral region. If the white-eyed animal is heterozygous for shot-veins (whwh.Svsv), the spots are reduced in extent. The variegated effect is therefore semi-dominant in respect to non-variegated. Variegated stock is fully fertile and stable. White and shot-veins have been combined with other mutant eye factors. Variegation shows in no combinations except with three allelomorphs in the orange series; type (O), dahlia (o^d), and orange (o) and with the carrot allelomorph to white. Wh.sv.O is typical variegated, wh.sv.o^d is light variegated, and wh.sv.o is very slightly variegated. White in combination with its allelomorph carrot and with shot-veins ($whwh^c$.svsv) produces typical variegation pattern on a cream background; with heterozygous shot-veins ($whwh^c$.Svsv) it gives slight variegation on cream background.

The dominance of shot-veins (and of its secondary effect, variegation) suggests that it is due to translocation but allows the possibility of its being a gene mutation. If due to translocation it must be stable since variegated is fully fertile even in haploid males and breeds true. Two suggestions are offered to explain the red spots. First: shot-veins factor or condition is associated with the constant habit of somatic mutation occurring at a definite stage in the development of the compound eye, such that only facets in the posterior ventral region are affected. Every eye is a mosaic (and a mosaic of rather constant pattern) according to this theory. Second: the shot-veins factor or condition has a spotting effect on the eyes when the residual heredity is such as to allow its expression and the cells of the red region are of the same genetic constitution as those of the white. The second theory is preferred by the author.

EGG-TRINUCLEARITY IN HABROBRACON. P. W. Whiting.—It has hitherto been assumed that mosaic males in *Habrobracon* develop from binucleate eggs in which the two nuclei correspond to the second polar nucleus and the reduced egg, the genetic difference between them resulting from post-reduction of those factors for which the mother was heterozygous. Several cases have now been obtained indicating egg-trinuclearity. Thus three of the four possible combinations of two pairs of allelomorphs have been shown in various parts of the body of the mosaic. At least three oötids from one oöcyte may therefore take part in parthenogenetic cleavage. Breeding tests have thus far shown not more than two of the possible combinations to be present in the sperm produced by the mosaic.

RELATION BETWEEN OXYGEN TENSION AND RESPIRATION IN SPIROSTOMUM AMBIGUUM, WITH CORRECTIONS FOR AMMONIA. Heinz Specht.—The rate of consumption of oxygen and of production of carbon dioxide in the ciliate, *Spirostomum ambiguum* (Saars), was measured under normal, low, and high tensions of oxygen by means of the Barcroft manometer (Gerard's modification, *A. J. P.*, 1931) following the technique of Warburg (*Bioch. Z.*, 1923 et seq.) with HCl in an additional chamber in the manometer vessel for the purpose of absorbing ammonia. Evidence is presented showing that ammonia is evolved, and that the correction for its evolution is essential in measuring the respiration of any tissue that produces it in appreciable quantities.

In air the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is respectively 2.59 $mm.^3 \pm 0.41$ $mm.^3$ and 2.17 $mm.^3 \pm 0.66$ $mm.^3$ when the evolved ammonia is absorbed by HCl. Without the acid the values are respectively 2.10 $mm.^3 \pm 0.9$ $mm.^3$ and 0.86 $mm.^3 \pm 1.23$ $mm.^3$. In an atmosphere of nitrogen containing 0.5 per cent oxygen the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is respectively 1.86 $mm.^3 \pm 0.95$ $mm.^3$ and 2.53 $mm.^3 \pm 0.81$ $mm.^3$ until disintegration of the animals sets in, then the values are respectively 0.07 $mm.^3$ ± 0.44 $mm.^3$ and 2.79 $mm.^3 \pm 2.04$ $mm.^3$. Without HCl the values under these conditions and over the same periods are respectively 0.76 mm.^{3} ± 0.28 mm.^{3}, 0.76 mm.^{3} ± 0.89 mm.^{3}, and 0.74 mm.^{3} ± 0.41 mm.^{3}, 3.24 mm.^{3} ± 1.06 mm.^{3}. This slight rise in the rate of production of carbon dioxide after disintegration sets in indicates a process which is not necessarily life-supporting, *i.e.,* not anoxybiotic but simple anoxic. In a concentration of oxygen of about 100 per cent the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is 3.92 mm.^{3} ± 0.79 mm.^{3} and 3.80 mm.^{3} ± 0.14 mm.^{3} respectively. This high rate is accompanied by a progressive injury to the animals and rises but slightly when they disintegrate. *Spirostoma* subjected to low tensions of oxygen for two hours and then furnished with oxygen at the normal tension exhibit a rise in the rate of respiration which approaches that in pure oxygen.

Increasing the oxygen tension 5 times over the normal value in air causes an increase to 1.5–1.7 times normal respiration, while decreasing it 40 times under the normal tension causes a decrease in consumption of oxygen to 0.7 times the normal value and an increase in production of carbon dioxide to 1.17–1.29 times the normal value.

It is concluded that the respiration of *Spirostomum* is nearly independent of oxygen tension down to very low values.

LUMINESCENCE AND RESPIRATION OF BACTERIA IN CARBON MONOXIDE. C. S. Shoup.—Carbon monoxide inhibits the respiration of luminous bacteria, but produces no significant loss of luminescence down to a decreased respiratory rate of only 45 per cent of an identical control suspension in $N_2 + O_2$ instead of $CO + O_2$. Luminescence and respiration are independent of each other in the presence of CO and low O_2-pressures down to the point of dimming due to oxygen-lack alone (0.26 per cent oxygen: Shoup, 1929). There is some evidence of a partial recovery from CO-narcosis.

THE RELATION BETWEEN LUMINESCENCE AND RESPIRATION IN BACTERIA WITH ESPECIAL REFERENCE TO THE EFFECTS OF NARCOTICS. G. Wellford Taylor.—If bacterial luminescence were dependent upon respiration, variations in the respiratory rate should be accompanied by similar variations in luminescence. This was not found to be the case in studying the effects of narcotics and certain other substances. In this work marine luminous bacteria were used as their own indices of rate of oxygen consumption by means of the dimming method of Harvey. Luminous intensities were measured on a photometer bench.

It was found that low concentrations of narcotic stimulated luminescence, higher concentrations produced reversible narcosis, while still higher concentrations caused irreversible changes. The same relation of effect to concentration holds for respiration but the concentrations are much higher than for luminescence, so that concentrations sufficient to markedly inhibit luminescence may stimulate respiration. In this way luminescence has been decreased 50 per cent while at the same time respiration increased 100 per cent.

A further separation of the two processes has been effected with KCN, with which respiration has been reduced 60 per cent without decreasing luminescence.

It has also been observed that when a fresh suspension of bacteria is allowed to stand, with aeration, its luminescence and respiration vary with time, but they do not vary together. The luminescence may increase 100 per cent without any increase in respiration; the respiration may decrease 45 per cent without affecting the luminescence.

The amount of nutrient material in a suspension is an important factor in determining the narcotic effect of respiration. By controlling this factor the stimulation of respiration by a given concentration of narcotic has been varied from 3 to 100 per cent, the stimulation increasing as the amount of nutrient decreased. This suggests that in addition to the stimulation of respiration per se, the narcotic may be used as food material by the bacteria. There is other evidence to support this view.

From these observations it appears that within wide limits luminescence is independent of respiration.

Nature of the Aerobic Apparent Reduction Potential. Lyle V. Beck.

The Relative Abundance of Hydrogen Isotopes in Sea Water. Eric G. Ball.—The ratio $H^2:H^1$ in Berkeley city water has been calculated by Lewis and Macdonald to be of the order 1:6500. Bleakney and Gould have given the ratio in rain water as 1:5000. The apparent inability of living cells to utilize water composed of the heavier hydrogen isotope (Lewis) demands the investigation of water from other natural sources for variations in this ratio. It seemed possible that an increase in the concentration of the heavier hydrogen isotope might occur with increasing depths in the ocean.

Two samples of sea water were employed. One sample, kindly supplied by Dr. A. C. Redfield, was collected in the Caribbean Sea at a depth of 4500–5000 meters during a recent voyage of the "Atlantis." The other sample was Woods Hole surface water. Distilled water was used for comparison. All samples were distilled five times from an all Pyrex still, freed from dissolved air, and the specific gravity determined in a 100 ml. pycnometer. The specific gravity of all three samples agreed within the experimental error of $\pm 5 \times 10^{-6}$. It therefore appears that the relative abundance of the hydrogen isotopes in sea water from the surface or from great depths is of the same order of magnitude as that previously reported for fresh water samples.

Toxicity of Some Metals and Berkefeld Filtered Sea Water to Mytilus edulis. Oscar W. Richards.—Three young (5–8 mm.) mussels were placed in a finger bowl with 100 ml. of sea water and the metal to be tested. The water was changed about every three days during the 41 days of the experiment and the temperature averaged 22° C. When an animal died it was replaced. In the following table of data the growth of the animals is expressed as a percentage of that of the control animals and the toxicity of the metals is given as the average time required to kill all three of the animals.

Source	Material	Type	Form	Growth	No. dead	Remarks
				per cent		
Control	—	—	—	100	1	—
Newark Wire Cloth Co.	Monel	—	screen	none	14	Kills in 1–2 days
Newark Wire Cloth Co.	Aluminium	pure	screen	100	none	No corrosion
Newark Wire Cloth Co.	Stainless steel	KA2-S	screen	106	1	No corrosion
Newark Wire Cloth Co.	Phosphor bronze	—	screen	none	9*	Kills in 20 hours. Highly corroded
International Nickel Co.	Inconel	(80% Ni, 14% Cr., 6% Fe)	strip	24	3	Slightly corroded
International Nickel Co.	Nickel	Finish No. 8	strip	30	4	No corrosion
International Nickel Co.	Monel	—	strip	none	11	Kills in 1–3 days
J. Bishop & Co.	Tantalum	—	spinnets	60	3	No corrosion
U.S.B.F.	Nickel	—	wire	83	1	No corrosion
Precision Sci. Co.	Brass	—	clamp holder	none	14	Kills in 2–3 days. Moderate corrosion
Fisher Sci. Co.	Castaloy	—	clamp holder	none	12	Kills in 3–4 days. Highly corroded
Berkefeld candle immersed in sea water	—	—	—	none	14	Kills in 2–3 days
50 ml. Berkefeld filtered sea H_2O + 50 ml. sea H_2O	—	—	—	18	2	—
75 ml. Berkefeld filtered sea H_2O + 25 ml. sea H_2O	—	—	—	1	4	—

* Discontinued after 19 days.

Berkefeld filtered sea water is not satisfactory for the dilution of available food in sea water for *Mytilus* in growth studies. Aluminium is non-toxic but not strong enough for the construction of live boxes. Stainless steel and nickel, although somewhat more expensive than the usual asphalt-covered galvanized iron, are non-toxic and could be used for the screen covering of live boxes. However, the former will rust slightly at the water line when it is not completely immersed in sea water.

SOME NEW ASPECTS OF THE NERVE-MUSCLE PHYSIOLOGY IN CRUSTACEA AS SHOWN BY TIME AND INTENSITY FACTORS IN ELECTRICAL EXCITATION AND RESPONSE. Herbert H. Jasper.*—Chronaxie values of 0.6 to 1.0 σ are obtained for the appearance of the first action potential in isolated claw nerves (crabs and lobsters). The apparent chronaxie, for a threshold (visible) contraction of the adductor claw muscle, ranges from 8 to 20 σ.

Nerve action potentials (with cathode ray oscillograph) show a rhythmic response to single constant current stimuli slightly above the intensity and time threshold for the appearance of a single wave. The frequency of nerve response (within certain intensity and time limits) is very regular for the complete duration of the current flow and increases with intensity up to the limit imposed by the refractory period. Threshold muscle contractions are observed only with stimuli which elicit a certain number and frequency of nerve response which explains the large discrepancies observed between nerve and muscle chronaxie measurements. The apparent "chronaxie" of isolated nerve muscle approaches a "temps de sommation" and cannot be called a chronaxie.

The experiments of Keith Lucas, which tended to show a slow and fast nerve-muscle system in *Crustacea,* have been repeated with most of the experimental facts being verified although extended in the light of the rhythmic response of the nerve to single stimuli and the consequent tetanic nature of muscle response which explain all the phenomena observed without the necessity of the hypothesis of a dual nerve-muscle mechanism. The slow and fast nerve action potentials found by Monnier and Dubuisson are probably due to the potentials from sensory and motor nerves and not to a double motor system since we have found that the slow wave is elicited upon sensory stimulation.

It was found that adductor and abductor claw muscles as well as nipper and crusher muscles in the lobster differed in their speed of contraction with corresponding differences in summation time and frequency for fused tetanos. Since these differences in rapidity of excitation and response seem to be associated with histological differences in myomere size, we draw the conclusion that excitation and inhibition in crustacean muscle as well as speed of response is a function of the intensity and time characteristics of the end organs; the latter being qualitatively not unlike all other striated muscle if the experiments of Bremer on summation in frog muscle are taken into account.

THE BLOOD PRESSURE OF LIMULUS. Margaret Sumwalt and Kathryn McLane. —In the frontal artery of *Limulus,* close to its origin from the heart, the most frequently observed mean blood pressure is 16 cm. H_2O. The range of all the variations observed among different individuals, or in the same individual with bouts of struggling, is included between 6 and 32 cm. H_2O. The pulse rate is between 15 and 39 beats per minute, and appears to be correlated inversely with the mean pressure.

The figures are based on study of 19 full grown unanæsthetized males. An animal fresh from running sea water is tacked through the margin of the carapace to a board. Removal of a square inch of the shell (usually without hemorrhage) exposes the artery. A cannula filled with mineral oil connects the artery with a mercury manometer, the meniscus of which is photographed, and with a straight erect oil manometer for calibration. (The ordinary anticoagulants are ineffective against the type of clotting which occurs in *Limulus* blood, whereas oil in the cannula does somewhat delay it.) The oil manometer is secondarily calibrated against a water manometer. Some of the data summarized above were obtained by direct observation of the oil manometer against a scale, some by analysis of photographic records. The error by either method does not exceed 2 cm. H_2O.

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* National Research Council Fellow.

The observations were made as a contribution toward a knowledge of the comparative physiology of water balance among the invertebrates. If the Starling hypothesis, which governs present ideas of water balance in the vertebrates, is also applicable to such of the invertebrates as possess a closed circulatory system, a direct relation should be found between the colloid osmotic pressure of their bloods and the systemic blood pressure. Very few measurements of either sort of pressure in an invertebrate are now on record.

THE EFFECT OF pH UPON POTASSIUM PENETRATION INTO FUNDULUS EGGS. John C. Bridges and Margaret Sumwalt.—Using standstill of the embryonic heart as a criterion for the presence of a definite concentration of K in the egg, we have found that increasing acidity, within the pH range down to about 3.6 where acid injury begins to occur, decreases the penetration of this cation. The time required to stop 50 per cent of the hearts with M/2 KCl is 60 minutes at pH 4, 38 minutes at pH 5, 36 minutes at pH 6, and 34 minutes at pH 7. Unbuffered solutions were used.

The results are interpreted to mean that acidity decreases the permeability of the egg to K. At least part of this increased permeability is believed to occur at the chorion. The relative rates of penetration of various ions through the chorion of the *Fundulus* egg have already been determined by the method of measuring potential differences. It appears that the chorion in neutral solutions is more permeable for cations than for anions, but that with increasing acidity its ion favoritism is gradually withdrawn and finally reversed. It would be interesting to know whether the withdrawal of membrane influence by acid permits an increased penetration of anions, or reduces the movement of cations, or does both to different extents; but the permeability of a membrane for ions in any absolute sense cannot be gauged by measurements of potential differences. For this particular cation, K, the present experiments indicate that acidity decreases the absolute penetration.

A METHOD FOR FOLLOWING VOLUME CHANGES OF CELLS. Arthur K. Parpart.

THE INFLUENCE OF pH UPON THE PASSAGE OF HEMOGLOBIN THROUGH THE GLOMERULUS OF THE PERFUSED FROG'S KIDNEY. William R. Amberson, Frank Engel, Dorothy Webster, and Edwin P. Laug.—Hemoglobin in solution in the blood stream of vertebrates leaves fairly rapidly through several exits. Most of it is lost through the kidney, and its disappearance leads to the death of animals whose normal blood has been replaced by hemoglobin-Ringer, a solution containing a quantity of hemoglobin from hemolyzed beef blood cells.

In an attempt to discover a method which may minimize or prevent the loss of hemoglobin into the urine, we have begun a study of various physico-chemical factors involved in the glomerular elimination of hemoglobin, and are now able to report on the influence of the H-ion concentration. We have perfused the kidneys of large bull frogs through aortic cannulæ, and have been able to secure readings relating the hemoglobin in the urine to that in the perfusion fluid over a pH range from 5.2 to 7.9. We find that hemoglobin passage is very much greater on the acid side of the isoelectric point of hemoglobin than on the alkaline side. The hemoglobin in the urine is about 10 per cent of that in the perfusion fluid, on the average, at pH 7.2 to 7.9, whereas it reaches about 30 per cent between pH 6.0 and 6.6, and rises to as high as 70 per cent at more acid values. The effect is reversible. Thus a high hemoglobin may be obtained with a perfusion at a low pH, to be succeeded by a low amount at a more alkaline value, in the same kidney. We have been able to reverse the effect up to five times.

We are inclined to explain the effect as being related to the charge of the hemoglobin ion, although a reversible change in the glomerular membranes is not ruled out. It seems possible to us that, on the alkaline side of its isoelectric point, the negatively charged hemoglobin ion may find it relatively difficult to pass through negatively charged glomerular membranes, but may penetrate with ease when its charge is reversed. If this is correct it suggests that other factors than the large size of the protein ions are involved in their penetration through living membranes.

THE INFLUENCE OF THE ESCAPE OF SALTS ON THE OSMOTIC BEHAVIOR OF THE ERYTHROCYTE. M. H. Jacobs and Arthur K. Parpart.—Certain deviations of the behavior of the erythrocyte from that of a perfect osmometer have been explained by Ponder and his collaborators as being due to an escape of salts from the cell. While such an escape is probable in experiments of sufficiently long derivation, it is shown that the osmotic properties of the erythrocytes of the ox, as studied by the hemolysis method, do not seem to undergo an appreciable change in times shorter than several hours. More specifically, the degree of hemolysis produced by a given hypotonic NaCl solution is not decreased by a preliminary exposure of from a few seconds to two or three hours to a slightly less hypotonic solution, in itself just unable to cause hemolysis. An increased osmotic resistance, suggestive of the escape of salts, is, however, produced by longer exposures. The properties of the erythrocytes of the pig, the cat, the rabbit, and man change more rapidly and these cells are therefore less favorable for certain types of osmotic studies than those of the ox.

In solutions of sucrose, either hypotonic or isotonic, there is a very rapid increase in the osmotic resistance of erythrocyte. This increase occurs in two stages: a very rapid one and a much slower one which follows the first after a considerable interval. While the slow change may well be associated with an escape of salts, the more rapid and more striking initial one is interpreted as the effect of an ionic exchange. In favor of this interpretation is not only the rapidity of the process but also its reversibility. The ionic exchange in question is believed to be one of anions from the cell for OH′ ions from the solution, the resulting increase in base bound by hemoglobin causing a decreased osmotic pressure within the cell.

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RATE OF GROWTH, AGE AT SEXUAL MATURITY, AND DURATION OF LIFE OF CERTAIN SESSILE ORGANISMS, AT WOODS HOLE, MASSACHUSETTS

B. H. GRAVE

(*From the Marine Biological Laboratory, Woods Hole, Mass., and DePauw University, Greencastle, Indiana*)

While studying the breeding habits and rate of development of *Teredo* the writer noted the very rapid growth of several sessile organisms, notably campanularian hydroids, *Hydroides*, *Balanus*, *Botryllus*, and *Molgula* which became attached to the wooden floats that were placed in the water in connection with the above study. No doubt the remarkably rapid growth of many animals of this sort has been observed by all who interest themselves in sea life. There are, however, comparatively few accurate data on the rate of development of these organisms and their age at sexual maturity. Studies of embryology have usually ended with organogeny rather than with life histories.

Without definite data it would not have been suspected that numerous organisms reach sexual maturity in three or four weeks or that the rate of multiplication and of growth is so remarkably rapid as it has proved to be. These facts contribute to an understanding of the sea as a reservoir of life and the methods by which this life is maintained.

In this paper the discussion of each species has been stated in the briefest possible form because we are here interested only in the outstanding facts of their life histories including the limits of the breeding seasons, rate of growth, duration of life, and number of generations per year. The study as a whole is intended to give a correct idea of the productivity of the organisms included. In this way a foundation is laid for an understanding of the interrelation of organisms and for greater accuracy in ecological or experimental studies.

This investigation was done at the Marine Biological Laboratory at Woods Hole and has covered a period of approximately ten years. During two years observations and measurements were made during every month in the year. Aside from this the work was confined to the summer months.

I wish to acknowledge my indebtedness to Dr. M. H. Jacobs, Director of the laboratory, and to the permanent staff for assistance and for facilities furnished during the course of the investigation.

METHODS

In studying the rate of growth of organisms certain materials and devices were finally selected to serve as places of attachment for sessile organisms. Among the most useful of these were wooden crosses constructed of 2″ × 4″ timber. These were placed in the Eel Pond and anchored to piles at dated intervals. The approximate date on which animals became attached could thus be known and the records of rate of growth kept with accuracy. The entire life history of individual animals could be followed or samples could be studied at intervals. Other materials that proved useful were 2″ × 4″ stakes ten or twelve feet long, one end of which was sharpened and driven into the mud while the other was nailed to a pile at the surface of the water. These and lobster pots made of smaller timbers proved to be most useful for the study of *Teredo*, but they served also as places of attachment for several other animals. Shells of mollusks, small stones, and bricks were sunk in wooden cages to serve expecially as attaching material for *Hydroides*. Finally, small wooden frames with grooves for holding microscope slides or small wooden strips served as materials easily examined by the microscope. These last were particularly valuable in observing early stages of metamorphosis and growth. Most animals which attached to microscope slides grew normally, but more slowly than when they attached to other materials. The floating wooden crosses were by far the most useful for the study of hydroids, barnacles, Bryozoa, and Ascidia. The sunken timbers were for *Teredo* and the stones and shells for *Hydroides*. In the discussion which follows, frequent reference will be made to the floating wooden crosses constructed as described above.

COELENTERATA

Most hydroids thrive best during spring and fall, although there are a few that do not reproduce until June. One or more of the four species studied were reproducing every month in the year at Woods Hole, with the possible exception of January. They, together with certain algae, remain active and growing throughout the winter and are clearly in healthiest condition while the water is cool. Although the growth of hydroids was comparatively slow during February, March, and April, the colonies and individual polyps appeared to be particularly healthy. They were free from debris and bacterial growth which so completely smothers them during July and August in such situations as the Eel Pond. They harbored a few stalked diatoms which themselves were clean and apparently no detriment. Chief among the enemies of hydroids at this time, as mentioned also by Coe, are nudibranch mollusks which feed upon the exposed polyps. From the middle of July until the middle of August hydroids are generally in very bad condition. The excessive growth of bacteria during the hottest part of the summer no doubt contributes to their hardships although they are distinctly cold water animals and are affected adversely by high temperature.

Two of the species of *Campanularia* studied, *C. flexuosa* and *C. calceolifera*, reach sexual maturity in four weeks under the most favorable conditions. This is also true of *Gonothyrea loveni*. All of them, under usual conditions, are reproducing abundantly in five weeks. The largest species studied, *Obelia commissuralis*, grows rapidly for six to eight weeks before reproducing sexually. Its colony when fully grown is four to five inches in length and consists of hundreds of polyps in contrast to the smallest species which have from twenty to fifty polyps. They all reproduce most abundantly in April and May and continue with little check until the middle or end of June, when they seem to disintegrate and although some new colonies are initiated throughout the summer, they are all in poor physiological condition. Late September and October are also periods of rapid growth. As cold weather comes on the rate of growth is reduced but the polyps remain active and new colonies continue to be established.

Although hydroids are not large animals they are so abundant that they foul all timbers at or near the surface during most of the year. Together with *Bugula* and *Botryllus*, which are abundant in summer and fall, they effectively cover all surfaces in a few weeks.

The planula larvae of species studied swim for a few hours and then become attached. Under favorable conditions they develop into feeding polyps within twenty-four hours. At this time they are already elongated and the hydrocaulus has a distinct perisarc including characteristic joints near the base. The rate of increase in growth of the colony varies with the species and with the temperature. In the following table the rate of growth of *Obelia commissuralis* is given. It shows that the number of polyps of the growing colony doubles every two or three days. After five days the first polyp which arises from the planula may have budded to a colony of five to seven feeding individuals, and in nine days there are twenty to forty polyps. The colony consists of hundreds or even thousands of individuals before it becomes sexually mature. The polyps were not counted in any colony more than a month old when it measured three inches in height and consisted of more than five hundred individuals.

So far as sexual reproduction is concerned, the smaller species proved to be actually more prolific than this species. They produce gonosomes early and the numbers of planuli produced by them during April and May is remarkably great.

The normal duration of life of hydroids varies with the time of year, but very few colonies survive the summer. The old colonies are so rapidly replaced by younger ones that it has been a difficult matter to get definite data in regard to length of life. It is certain that they live for several months unless difficult conditions arise. They certainly do not survive for a year. The total amount of growth is, however, great in situations favorable to their development.

TABLE I

Rate of growth of Obelia commissuralis. The left-hand column gives the period of growth beginning with the attachment and metamorphosis of the planula. The column at the right indicates the year. All measurements are of actual colonies and are not averages.

Period of Growth	Age	Height	Colony	Year
		mm.		
June 24	0 day	.35 × .10	Planula	1926
June 24 to June 25	1 day	.8 × .07	1 polyp	1926
June 20 to June 25	5 days	2 to 3	5 to 7 polyps	1932
June 19 to June 27	8 days	8.5 to 10	28 to 40 polyps	1931
June 9 to June 18	9 days	14 to 17	26 to 42 polyps	1931
June 9 to June 19	10 days	15 to 17	50 to 65 polyps	1931
June 20 to June 30	10 days	19 to 20	55 to 65 polyps	1932
June 9 to June 22	13 days	22 to 26	140 to 160 polyps	1931
June 10 to June 24	14 days	30	140 to 160 polyps	1932
June 9 to June 27	18 days	55 to 57	500 to 560 polyps	1931
April 8 to May 18	40 days	45 to 50		1927
Aug 12 to Oct. 12	60 days	65 to 70		1926
Oct. 10 to Jan. 3	86 days	100 to 120		1926
Jan. 1 to May 15	4½ months	50 to 55		1926 and 1927
Sept. 20 to May 15	Winter	55 to 65		1926

CRUSTACEÆ—BALANUS EBERNEUS

Of the several species of barnacles that occur at Woods Hole, *Balanus eberneus* is the only one which thrives in the Eel Pond, where this study has been conducted.

The eggs are retained in a brood cavity during early development and the young issue as nauplius larvæ which swim actively. These have a free-swimming period of approximately a week or ten days during which they moult several times and pass through the transformation stages known as nauplius, metanauplius, and cyprid. The cyprids finally settle upon solid objects such as stones, piles and wooden structures and metamorphose into the adult form. The present study is concerned principally with the breeding season and rate of growth from metamorphosis to adult size. My conclusions concerning the duration of the free-swimming nauplius are based upon the observation that after the first appearance of swimming larvæ only a week elapses before metamorphosis and permanent attachment of the cyprids take place.

TABLE II

Rate of Growth of Balanus eberneus

Period of growth	Age	Length	Breadth	Year
		mm.	*mm.*	
July 19 to July 25	6 days	.8	.7	1932
Aug. 8 to Aug. 17	9 days	2	1.7	1929 and 1932
June 27 to July 18	10 days	2.3	2.	1930
July 15 to July 27	12 days	2.8	2.3	1926
July 1 to July 15	14 days	3.5	2.8	1926
Aug. 4 to Aug. 18	14 days	3	3	1929
June 24 to July 13	16 days	4	3.5	1930
July 25 to Aug. 12	18 days	5.2	4	1926
July 15 to Aug. 12	28 days	7.4	6.5	1926
June 20 to July 22	32 days	10	9	1932
June 22 to July 29	32 days	9	8	1927 and 1931
July 15 to Aug. 20	36 days	10	9	1926
June 27 to Aug. 2	36 days	11	10	1930
June 14 to July 22	38 days	12	11	1932
July 9 to Aug. 23	44 days	13	12	1927
June 27 to Aug. 15	49 days	15	14	1932
June 27 to Aug. 15	49 days	15	13	1926
June 27 to Aug. 20	54 days	16	15	1930
June 27 to Aug. 20	54 days	17	16	1931
June 23 to Aug. 23	60 days	15	14 *	1927
June 23 to Aug. 23	60 days	14.5	12.5*	1925
July 15 to Sept. 20	67 days	17	13	1926
June 27 to Sept. 7	76 days	15	14	1927
June 20 to Sept. 4	70 days	19	16	1931
July 1 to July 1	1 year	20.5	19.5	1927
July 1 to July 1	1 year	18	16	1926

* Sexually mature in 60 days and over.

According to data collected over a period of years the first larvæ of the season escape from the parent between June 7 and June 15. The first cyprids become permanently attached and metamorphose between June 15 to June 23. The first young barnacles appear visible to the unaided eye between June 23 to July 4. Ten days after metamorphosis the young barnacles measure 2 × 1.5 mm. and are readily seen upon the wooden floats used in this study.

From the time the cyprid larva metamorphoses into the barnacle the growth is rapid as shown by Table II. Sexual maturity is reached in two months (60 days) and the first nauplius larvæ issue from young barnacles of the season between August 20 to August 25. For example, a float placed in the Eel Pond on June 23 had barnacles attached which extruded nauplius larvæ on August 23. There are considerable numbers of barnacle larvæ in the water from August 25 to October 1, but the fall breeding season amounts to little compared with the June and July breeding season of this species.

Growth practically ceases late in October and is resumed the following spring.

At the end of one year this barnacle measures from 18 × 16 mm. to 21 × 19 mm. depending upon conditions, including crowding. They apparently reach maturity during the second year. No individuals have been kept under observation for a longer period than one and one-third years.

Although this species of barnacle breeds more or less all summer, the important part of the period of fouling extends from June 25 to July 25, or from June 25 to July 15. By far the greatest amount of fouling of timbers, therefore, occurs during a period of three weeks. Those attaching after July 25 are negligible. This was shown to be true also of a winter breeding species. The cyprids of *Balanus balanoides* metamorphose in greatest numbers between February 15 and March 15, and although they continue to shed a few nauplius larvæ until May, the breeding season is mainly over in four weeks.

In general, barnacles reach sexual maturity early and are short-lived as compared with many other crustaceans.

ANNELIDS—HYDROIDES HEXAGONIS

This species of annelid secretes a calcareous conical tube which adheres to shells of mollusks, stones, and wooden structures.

The breeding season opens between June 10 and June 20 and closes between October 20 and November 1. The egg has an unusually heavy vitelline membrane. It measures from .067 to .072 mm. when fully mature. During the early part of the breeding season over fifty per cent of the spawned eggs are immature and undersize. Toward the end of the breeding season the eggs are all mature, indicating that the germinal epithelium is becoming inactive. Maturation of the egg does not take place until the spermatozoön enters. The germinal vesicle measures half the diameter of the egg or (.03 mm.). The sexes are separate. To obtain eggs or sperm, it is advisable to remove the worms from their calcareous tubes and place them in dishes of sea water, one worm per dish. When so treated they always spawn immediately. The spermatozoa when first extruded are immobile but become slightly activated after remaining in sea water for approximately half an hour. It is a relatively inactive sperm at best. After fertilization the egg develops within twenty hours into a spherical transparent trochophore larva, which swims for at least ten days before metamorphosis.

It finally and gradually develops a slender worm body, settles and secretes a calcareous tube. After this the worm is immovably fixed to some supporting object. In studying the rate of growth the increase in the size of the worm tube is taken as the index of the growth of the worm. The shell, of course, grows and extends in length from its open mouth. It is not a difficult matter to study the growth of a single worm, but in practice the largest worms of certain ages were measured, and no attempt was made to record the growth rate of a single worm. The data for the several years of study is fully consistent except that the growth rate for the years 1924–25 is not equal to that observed during the past three years.

Table III gives the approximate rate of growth after metamorphosis.

TABLE III

Rate of Growth of Hydroides hexagonis

Period of growth	Age	Length and breadth	Year
		mm.	
Aug. 6 to Aug. 22	16 days	7 × .6	1931
July 20 to Aug. 10	21 days	11 × .6	1927
July 20 to Aug. 15	25 days	13 × .8	1931
July 20 to Aug. 20	30 days	20 × 1.2	1931
Aug. 2 to Sept. 3	33 days	13 × 1	1931
June 20 to Aug. 2	36 days	25 × 2	1924
June 27 to Aug. 11	44 days	37 × 2.2	1931
June 27 to Aug. 15	49 days	45 × 2.3	1931
June 27 to Aug. 25	59 days	54 × 2.7 *	1931
Oct. 18 to May 6	Winter	5 × 1.1	1926
Aug. 20 to Aug. 20	1 year	66 × 3.0	1924 and 1925
	2 years	120 × 5	1927

* Sexually mature in 59 days.

The worm becomes sexually mature in seven or eight weeks after metamorphosis, and before it is nearly half grown. The first eggs from these young worms are spawned about August 20, and egg production continues until about October 25 in both young and old worms. The larvæ of this species are metamorphosing and worm tubes are being formed during the entire breeding season. The worm becomes fully grown in two years and probably does not live beyond that age. The tubes of the largest worms measure 12 cm. in length and 5 mm. in greatest diameter. A worm 10 cm. long and 4.5 mm. in widest diameter is a large worm and is nearly the average size at maturity. The largest of those kept for one full year measured 7 cm. in length and 3 mm. in widest diameter, which may be considered average. It should be said that no worms were kept under observation for more than fifteen months. Because they have not attained full size in that time the impression was gained that individuals survive for two years and not much beyond that. Most of the worms ordinarily collected are only one year old. Comparatively few survive to reach the largest size recorded in Table IV.

TABLE IV

Rate of Growth of Botryllus gouldii

Period of growth	Age	Size of colony *mm.*	Number of individuals of colony	Year
July 19 to July 22	3 days	?	One gonozoid complete	1932
July 19 to July 24	5 days	1.5 × 1.5	Gonozoid and first blastozoid complete and initiation of further budding	1932
July 19 to July 26	7 days	4 × 4	4 to 5 complete individuals and initiation of further budding	1932
July 19 to July 29	10 days	5 × 5	8 to 10 individuals	1931
July 20 to July 31	11 days	6 × 6	18 to 20 individuals	1931
July 20 to Aug. 3	14 days	10 × 7	30 to 45 individuals	1931
July 20 to Aug. 5	16 days	12 × 10	52 to 58 individuals	1931
Aug. 6 to Aug. 30	24 days	30 × 20	200 to 300 individuals	1927
Aug. 6 to Sept. 6	30 days	50 × 30	1000 individuals	1927
June 20 to July 20	30 days	90 × 50*	3000 individuals	1931
Oct. 10 to Nov. 26	Fall	5 × 5	6 to 8 individuals	1926
Oct. 26 to Jan. 3	Fall	5 × 5	6 to 10 individuals	1926 and 1927
Oct. 26 to May 15	Winter and spring	12 × 12	50 to 75 individuals	1926 and 1927

*Sexually mature in 30 days and above.

ASCIDIANS—BOTRYLLUS GOULDII

The compound ascidian *Botryllus gouldii* has proved to be extremely variable in rate of growth. In compiling the table on rate of growth I have arbitrarily selected the figures which show the most rapid development observed in the eight years study. The data are taken mostly from observations of the same year. The table shows that the number of individuals of the growing colonies doubles every two or three days. Under favorable conditions a colony starting from a tadpole may consist of one or two thousand individuals in a month. The measurements as given are of representative colonies and not averages.

During August and September all floating and slightly submerged structures may be completely covered by the colonies of this species Growth during the cooler months slows down to insignificant proportions. The breeding season begins sometimes as early as May 15 or sometimes not until June 10. It continues until November 1. *Botryllus* has a long breeding season, but is most prolific during July, August, and September. The colonies reach sexual maturity and extrude tadpoles in from 35 to 50 days after metamorphosis. A wooden float placed in the water on June 20, 1931 developed colonies from single tadpoles which measured 9 by 5 centimeters, containing 3000 individuals on July 20. These colonies which were thirty days old contained mature eggs and on their thirty-fifth day, July 25, they extruded tadpoles. Another colony which developed on a float which was placed in Eel Pond on June 27 extruded swimming tadpoles on August 3. Another starting on May 15 became sexually mature on July 4. The species therefore reaches sexual maturity under the most favorable conditions in five weeks. Many large colonies survive and are active all winter, contracting perceptibly when stimulated. However, a large percentage must die during the winter because in the spring there are no such numbers surviving as covered piles and floating structures during late summer. The chief fouling of these timbers occurs from June until October, after which the rate of growth and of reproduction is greatly reduced or practically ceases. Growth revives in March and reproduction in May. A few individuals surviving the winter might literally populate to capacity the adjacent parts of the sea suitable to their existence before the next winter. In establishing the colony the metamorphosing tadpole soon develops into a sessile individual, termed a gonozoid, similar to a minute molgula. The colony is then produced by characteristic and repeated budding, the details of which have recently been reinvestigated by Sister Florence Marie Scott at Woods Hole (not yet published).

MOLGULA MANHATENSIS

The growth of the solitary ascidian, *Molgula manhatensis*, may be given briefly by way of comparison with the colonial form. The ascidia are hermaphroditic and the eggs of this species are laid and fertilized at night. They develop within twenty-four hours into swimming tadpoles which soon become permanently attached. The attached tadpole metamorphoses promptly into the adult form and rapid growth ensues. When the molgula is two weeks old the gonads are visible through the translucent test. At three weeks of age a few mature eggs are shed and a larger number at a month. *Molgula manhatensis*, therefore, under favorable conditions reaches sexual maturity in three weeks or a month, at which time it measures approximately 10 × 8 mm. It spawns abundantly during its first season. A large specimen measures 23 × 22 mm., a size which may be attained before winter. To secure eggs, place sexually mature specimen in a dish of sea water and allow to stand undisturbed. Eggs will be shed normally during the evening. They may also be had at any time by dissecting the gonads of sexually mature specimens.

BRYOZOA—BUGULA FLABELLATA

The numerous species of Bryozoa which occur along the Massachusetts coast are similar in the rapidity with which they grow and come to sexual maturity. Several generations are produced every season.

The breeding season of *Bugula flabellata*, taken as an example, opens between June 1 and June 15 and closes about November 15. With the exception of hydroids and possibly one or two ascidians, the Bryozoa are the only sessile animals that have been found breeding at Woods Hole after November 1.

Most of the larger colonies of *B. flabellata* are killed during the winter but the small ones survive and the species is abundantly reestablished in June. Sometimes the first new colonies are seen during the first week in June and sometimes not until the third week. Following a hard winter the breeding season opens late, which is correlated with the killing of the larger colonies.

Colonies of *Bugula flabellata* which are established in June reach sexual maturity in one month and old age in three months. Those which are established during October may survive for ten months including the winter, which is a period of hibernation. The life history of this species, including a table on the rate of growth, has been published (Grave, B. H., 1930). The only alterations to be made in that account are that the first avicularium appears on young colonies of four functional individuals rather than later, and when the colony has grown to eight functional individuals there are avicularia on the second, third, and fourth members of the colony. As stated before, the first or attaching member of the colony never develops an avicularium.

The hold-fast very early becomes branching and stolon-like rather than disc-like as stated in that paper.

The embryos of *Bugula flabellata* are developed in brood pouches or ovicells. The larvae are shed at dawn or early morning.

To secure swimming larvæ several mature *Bugula* colonies should be collected late in the afternoon and placed in a dish of sea water. Larvæ escape from these colonies on the following morning, often by hundreds. After swimming from four to six hours they become attached and the colony rapidly develops.

MOLLUSCA

Observations on three species of mollusca, *Teredo navalis*, *Cumingia tellinoides*, and *Chætopleura apiculata*, form an integral part of this study, but in each case separate articles have been published, which give the life histories of these forms in detail (Grave, B. H., 1928 and 1932). Mention should be made that *Teredo navalis* becomes sexually mature in two months and reaches adult size in one year: *Cumingia tellinoides* and *Chætopleura apiculata* become sexually mature in one year and reach adult size in four years.

SUMMARY

Although *Balanus*, *Hydroides* and *Teredo* become sexually mature and spawn during their first season when they are only two months old, they spawn most abundantly during their second season when they are one year of age. Each of them spawns several times during the second summer.

Bugula, *Botryllus* and various hydroids, on the other hand, spawn principally before they are two months old and it is doubtful if they ever survive for a full year. Some of the most prolific animals seem not to live more than a year and they owe their great abundance to their production of several generations in a single summer.

These characteristics of organisms in a measure account for the abundant life of the sea and of the rapid rehabilitation of certain species after depletion. Although the sessile animals studied do not survive for more than one or two years such animals as *Cumingia*, *Chætopleura*, and *Ostrea* often live from four to six years and spawn several times each year.

The surprising discovery of this study was the great rapidity of growth and early sexual maturity of these organisms.

BIBLIOGRAPHY

BAKER, F. C., 1911. The Lymnæidæ of North and Middle America. Chicago Acad. Nat. Sci. (Special Pub. No. 3).

BAILY, JOSHUA L., 1931. Some Data on the Growth, Longevity and Fecundity of Lymnæa columella. *Biologia Generalis*, Leipsig, **7**: 407.

BIGELOW, M. A., 1902. A Study of Cell-Lineage and Germ Layers. Early Development of Lepas. *Bull. Mus. Comp. Zoöl. Harvard College*, **40**: 61.

COE, WESLEY R., 1932. Season of Attachment and Rate of Growth of Sedentary Marine Organisms at the Pier of the Scripps Institution of Oceanography, La Jolla, Cal. *Bull. Scripps Inst. of Oceanography*, **3**: 38.

COKER, R. E., 1921. Natural History and Propagation of Fresh Water Mussels. *Bull. U. S. Bur. Fish.*, **37**: 75.

CRABB, E. D., 1929. Growth of the Pond Shell Lymnæa stagnalis appressa, as Indicated in Shell-Size. *Biol. Bull.*, **56**: 41.

FISH, CHARLES J., 1925. Seasonal Distribution of the Plankton of the Woods Hole Region. *Bull. U. S. Bur. Fish.*, **41**: 91.

GRAVE, B H., 1924. Rate of Growth and Age of Sexual Maturity of Certain Sessile Organisms. *Anat. Rec.*, **29**: 90.

GRAVE, B. H., 1927. The Natural History of Cumingia tellinoides. *Biol. Bull.*, **53**: 208.

GRAVE, B. H., 1928. Natural History of Shipworm, Teredo navalis, at Woods Hole, Massachusetts. *Biol. Bull.*, **55**: 260.

GRAVE, B. H., 1930. The Natural History of Bugula flabellata at Woods Hole, Massachusetts, Including the Behavior and Attachment of the Larva. *Jour. Morph.*, **49**: 355.

GRAVE, B. H., 1932. Embryology and Life History of Chætopleura apiculata. *Jour. Morph.*, **54**: 471.

JUST, E. E., 1922. On Rearing Sexually Mature Platynereis megalops from Eggs. *Am. Nat.*, **56**: 471.

LEFEVRE, GEORGE, AND W. C. CURTIS, 1910. Studies on the Reproduction and Artificial Propagation of Fresh-Water Mussels. *Bull. U. S. Bur. Fish.*, **30**: 109.

MEAD, A. D., 1899. The Natural History of the Star-fish. *Bull. U. S. Bur. Fish.*, **19**: 203.

NELSON, T. G., 1921. Aids to Successful Oyster Culture. *N. J. Agr. Exper. Sta. Bull.*, No. 351.

ORTON, J. H., 1920. Sea-Temperature Breeding and Distribution of Marine Animals. *Jour. Mar. Biol. Assn.*, N. S., **12**: 339.

VISSCHER, J. PAUL, 1927. Nature and Extent of Fouling of Ships' Bottoms. *Bull. U. S. Bur. Fish.*, **43**: 193.

VISSCHER, J. PAUL, 1928. Reactions of the Cyprid Larvæ of Barnacles at the Time of Attachment. *Biol. Bull.*, **54**: 327.

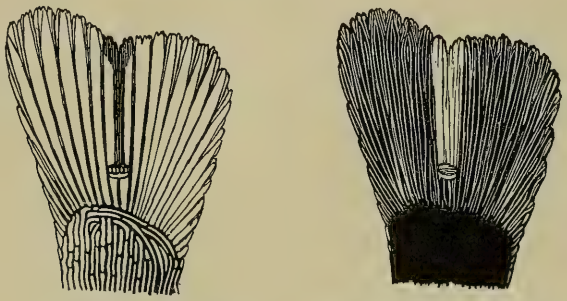
WEYMOUTH, F. W., H. O. MCMILLAN, AND H. B. HOLMES, 1925. Growth and Age at Maturity of the Pacific Razor Clam, Siliqua patula (Dixon). *Bull. U. S. Bur. Fish.*, **41**: 201.

THE ACTION OF ERGOTAMINE ON THE CHROMATOPHORES OF THE CATFISH (AMEIURUS NEBULOSUS)

Z. M. BACQ[1]

(*From the Marine Biological Laboratory, Woods Hole, Mass.*)

It is a well-known fact that ergotamine paralyzes the action of adrenalin and the effects of sympathetic stimulation. But ergotamine has also a definite action on the denervated cells, and this action, as far as it has been investigated, is the same as that of adrenalin. Thus ergotamine, in the cat, increases the rate of the denervated heart (Cannon and Bacq, 1931), contracts the denervated nictitating membrane (Rosenblueth, 1932 and personal observations), contracts the retractor penis muscle (Bacq, 1933), excites the secretion of the denervated sweat glands (Fröhlich and Zak, 1932 [2]), increases the blood pressure after complete sympathectomy (Bacq, Brouha, and Heymans, 1932).



I.–Before ergotamine.   II.–After ergotamine.

FIG. 1. Sketches of the tail of a catfish six days after cutting, before and after the injection of ergotamine.

Adrenalin contracts the melanophores of the fishes, either in normally innervated areas or in denervated regions. I found that ergotamine expands the chromatophores in the normal skin and contracts these cells when denervated. Catfishes (*Ameiurus nebulosus*) were kept in a white illuminated dish. A large cut was made in the tail according to the technique extensively used since Wyman (1924). A dark band due to the expansion of the chromatophores developed posterior to the cut. Six days after the operation, only the central portion of this band and a triangle at the posterior edge of the fin were dark. At that time, 0.25 mg. of ergotamine tartrate (Gynergen Sandoz) injected in the muscles would evoke after ten to fifteen minutes a complete darkening of the animal with the exception of the denervated area of the tail which appeared perfectly light. The dark band and triangle were light colored and indistinguishable from the rest of the denervated area. This condition persisted for two or three hours (Fig. 1).

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[1] Advanced Fellow of the C. R. B. Educational Foundation.

[2] These authors have found that adrenalin excites the secretion of the denervated sweat glands. I have been able to confirm their observations (Bacq, 1933).

These observations show that ergotamine expands the normally innervated chromatophores and that it contracts the same cells when denervated. This difference in reaction occurs also in the mammal. For instance, ergotamine decreases the blood pressure of the normal cat, but produces in this animal when sympathectomized a tremendous hypertension. This denervation of the smooth muscles of the arterioles shows a direct action of ergotamine on these cells.

CONCLUSION

Ergotamine expands the innervated chromatophores of the catfish and contracts the same cells when denervated. This effect on the innervated chromatophores is opposite to that of adrenalin, but the direct action of ergotamine and adrenalin on the denervated areas is similar.

BIBLIOGRAPHY

Bacq, Z. M., 1933. Recherches sur la physiologie du systeme nerveux autonome. III. *Arch. internat. de physiol.*, **36**: 167.

Bacq, Z. M., L. Brouha, and C. Heymans, 1932. Réflexes vaso-moteurs d'origine sino-carotidienne chez le chat sympathectomisé. *Compt. rend. Soc. Biol.*, **111**: 152.

Cannon, W. B., and Z. M. Bacq, 1931. Studies on the Conditions of Activity in Endocrine Organs. XXVI. *Am. Jour. Physiol.*, **96**: 392.

Frölich, A., and E. Zak, 1932. Untersuchungen über die periphere Schweissekretion. *Arch. f. exper. Pathol. u. Pharmakol.*, 620.

Rosenblueth, A., 1932. The Action of Certain Drugs on the Nictitating Membrane. *Am. Jour. Physiol.*, **100**: 443.

Wyman, L. C., 1924. Blood and Nerve as Controlling Agents in the Movements of Melanophores. *Jour. Exper. Zool.*, **39**: 73.

EFFECTS OF CENTRIFUGAL FORCE ON FERTILIZED EGGS OF ARBACIA PUNCTULATA AS OBSERVED WITH THE CENTRIFUGE–MICROSCOPE

ETHEL BROWNE HARVEY

(From the Marine Biological Laboratory, Woods Hole, Mass., and the Biology Department, Princeton University)

By means of the centrifuge-microscope of Harvey and Loomis (1930), it is possible to observe continuously the effects of centrifugal force on eggs at various stages of development, while they are being rotated. A striking difference in behavior of the fertilized and unfertilized eggs of *Arbacia* is immediately apparent when studied in this way—a difference connected with changes in the elasticity of the fertilization membrane, in the egg surface, and in the viscosity of the egg itself. Behavior of the unfertilized eggs has been previously described (E. B. Harvey, 1932, 1933), and this paper presents the phenomena to be observed after fertilization. The experiments were performed at a temperature of approximately 23° C. Observations have also been made on eggs centrifuged in tubes where higher speeds could be obtained.

FERTILIZED EGGS

When *Arbacia* eggs are fertilized and placed as quickly as possible (30–90 seconds) in the special slide for the centrifuge-microscope (or in tubes), above a layer of 0.95 molal sucrose solution (which keeps them suspended) and subjected to a centrifugal force of 5000 times gravity, they break apart very readily into halves and quarters (Photograph 1). If broken apart (in tubes at 11,000 times gravity) before the elevation of the fertilization membrane, this membrane may subsequently form over each part, but is thinner (ruptures more easily) over the centripetal pole of the part; it is also thinner over the oil cap of the elongate whole egg. These parts are similar to those from unfertilized eggs (E. B. Harvey, 1932), but in most batches of eggs the elongation of the egg is greater and the halves and quarters are produced much more rapidly, as shown by comparison with a control unfertilized lot centrifuged at the same time (Photograph 2). This must be due to a decrease in the tension of the surface and not to a decrease in the viscosity of the egg protoplasm, since *the fertilized eggs at this stage and at every other stage stratify less rapidly than unfertilized eggs.* This fact can be very accurately determined by observation of the two sets of eggs at the same time while rotating, in the special double-head centrifuge-microscope devised by E. N. Harvey (1933). The difference in stratifying between unfertilized eggs and eggs immediately or shortly after fertilization is very slight, but becomes very marked in later stages, as observed by Heilbrunn (1915, 1928) and Chambers (1919).

For about five minutes after fertilization, the fertilization membrane, which appears one and one-half to two minutes after insemination, is easily broken by the centrifugal force; and during this period (one and one-half to five minutes after fertilization) the eggs break very readily into many small pieces (Photograph 3 as contrasted with the unfertilized control in Photograph 2). Here again the viscosity as determined by stratification is less in the unfertilized egg.

After five minutes the fertilization membrane becomes so tough that it does not stretch and permit the eggs to elongate and break apart. It is possible, however, to remove the fertilization membranes as soon as they are formed by shaking the eggs quite violently for a moment. When the eggs from which membranes have been removed are centrifuged from five to twenty minutes after fertilization, during the monaster stage, they stretch out enormously, forming long streamers connecting clear and yolk portions of the egg. They stretch the whole length of the field, sometimes becoming ten times as long as the original diameter of the egg (Photograph 4). When these streamers break, they form beaded chains which retract into tails. If the centrifuge is

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PLATE I

PHOTOGRAPH 1. Fertilized eggs centrifuged 30 seconds after fertilization; note greater elongation of yolks and greater number of eggs pulled apart as compared with the control unfertilized eggs centrifuged at the same time (Photograph 2).

PHOTOGRAPH 2. Control unfertilized eggs centrifuged at same time and rate as Photographs 1 and 3.

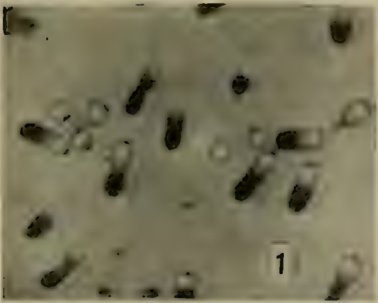
PHOTOGRAPH 3. Fertilized eggs centrifuged 4 minutes after fertilization. Note eggs broken into many small pieces and compare with control unfertilized eggs of Photograph 2.

PHOTOGRAPH 4. Eggs centrifuged 12 minutes after fertilization for 8 minutes; fertilization membranes previously removed, except for a few eggs which may be observed still nearly spherical. Photograph taken while rotating in centrifuge-microscope.

PHOTOGRAPHS 5 and 6. Retraction of eggs with slowing down of centrifuge; eggs centrifuged 10 minutes after fertilization; Photograph 5 taken 6 minutes later at high speed; then speed lowered and Photograph 6 taken 6 minutes later, same eggs. Note shortening and bending at bottom in Photograph 6.

PHOTOGRAPH 7. Eggs centrifuged 20 minutes after fertilization. Picture taken 12 minutes later while rotating. Elongate dumb-bells.

PHOTOGRAPH 8. Eggs cleaving while being rotated in centrifuge. Eggs (membranes not removed) rotated 3 minutes after fertilization at high speed (5000 × gravity) for 15 minutes, then low speed (2500 × gravity) for 1 hour. Photograph taken while rotating.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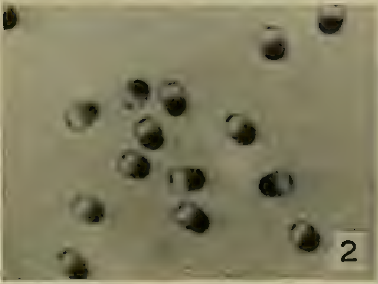
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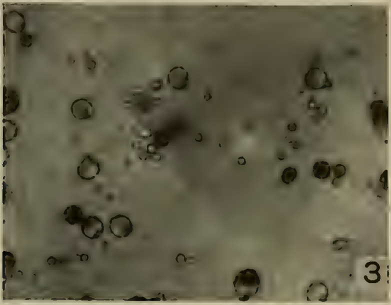
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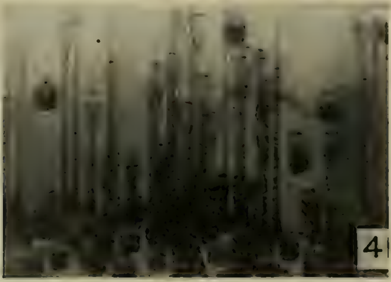
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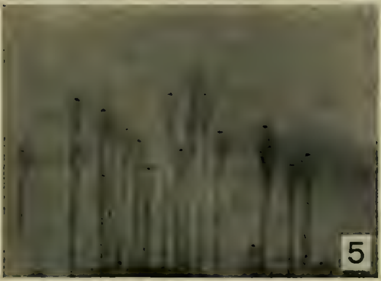
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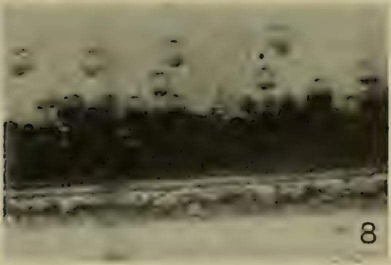
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slowed down at the stage of the long streamers, these contract and bend at the bottom, being evidently quite elastic (Photographs 5 and 6, same eggs). On increasing the speed, they again elongate slightly. If the centrifugal force is removed during the streamer stage, the greatly stretched eggs may contract into spheres or they may break in one or many places and these pieces become spherical. The formation of long streamers cannot be connected with the ectoplasmic layer since this is thrown off to the centrifugal pole as a crescent, easily seen on eggs with broken fertilization membranes—a phenomenon to be described in detail in a later paper.

After about 20 minutes after fertilization, during the streak stage, the eggs when centrifuged form shorter streamers or become elongate dumb-bells (Photograph 7) and break apart less and less readily as the interval after insemination increases. In some batches of eggs, the fertilized eggs break apart at all stages (provided the fertilization membranes have been removed) more readily than the unfertilized. In other batches they break apart less readily in later stages, or do not elongate much or break apart at all with the same centrifugal force. The stage at which the fertilized eggs break less rapidly than the unfertilized differs in different batches. This resistance to breaking can be overcome by keeping the eggs after removal of the membranes in calcium-free sea water. They then elongate when centrifuged and break apart readily at any stage up to cleavage. No difference in viscosity could be detected in (unfertilized) eggs kept in artificial sea water *without* calcium (brought to the same pH as sea water by the addition of sodium bicarbonate) and those kept in similar artificial sea water *with* calcium; a comparison was made of the rate of stratification in these solutions with the double-head centrifuge-microscope. The effect of the calcium-free sea water must therefore be a surface effect, as might be expected from its well-known influence on the ectoplasmic layer, causing its disintegration and the falling apart of blastomeres (Herbst, 1900).

The general striking differences in behavior of the fertilized (in

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PLATE II

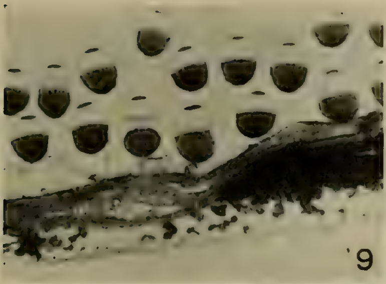
PHOTOGRAPH 9. Eggs centrifuged slowly (2500 × gravity) while fertilization membrane was being formed; two spheres inside membrane (lower left center egg). Photograph taken while rotating in centrifuge-microscope.

PHOTOGRAPH 10. Same removed from centrifuge-microscope. White sphere of upper egg has cleaved.

PHOTOGRAPH 11. Same. Development of white sphere, no development of red.

PHOTOGRAPH 12. Same. Development of both spheres.

PHOTOGRAPHS 13–16 (same egg). Same. Fusion of two spheres at first (Photograph 13) well separated within fertilization membrane.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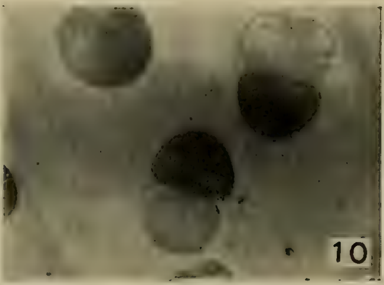
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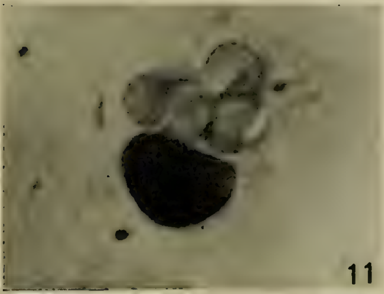
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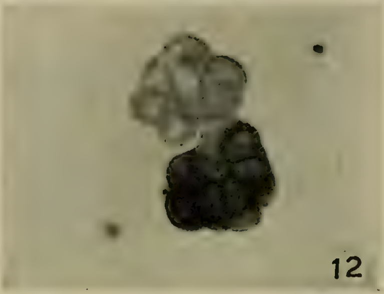
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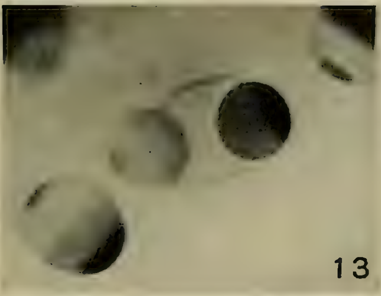
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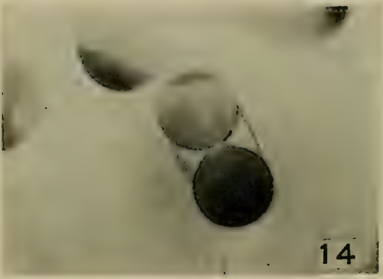
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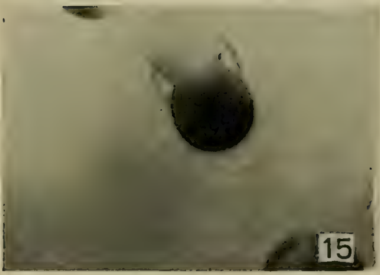
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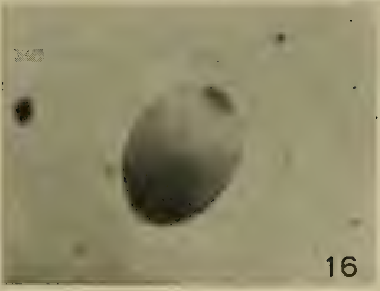
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various stages) and unfertilized eggs of *Arbacia* must be due primarily to surface changes. The more rapid breaking of the just fertilized eggs when centrifuged in spite of increased viscosity must be due to a decrease in surface forces. Cole and Michaelis (1932) have measured the surface forces of unfertilized and fertilized *Arbacia* eggs, but not during the period soon after fertilization when the breaking is most rapid; they found in fertilized eggs without fertilization membranes that there was no increase in surface forces until shortly before cleavage—a period when the eggs have become more difficult to break apart. At this stage it is possible that the great increase in viscosity may also be involved in the more difficult pulling apart. The great elasticity of the eggs at the streamer stage must also be connected with surface changes. Surface changes upon fertilization are indicated in the normal egg by the formation of the fertilization membrane and of the ectoplasmic layer, and the change in position of the pigment granules.

It is possible to break the egg apart into two spheres inside the fertilization membrane (Photographs 9, 10, 13); this is done by centrifuging the eggs immediately after fertilization but more slowly, with a force of 2500 times gravity. The fertilization membrane is frequently much stretched—from a diameter of 82 μ to a length of 128 μ. If the centrifugal force is now removed, either (1) the stretched membrane contracts, and the two spheres are brought into contact and gradually coalesce (Photographs 13–16, showing stages in complete coalescence) or (2) the two spheres remain separate and develop separately (Photographs 10–12). Since the sperm may enter the egg at any point, it may be in either sphere at the time of breaking apart. If the sperm is in the white sphere, it cleaves, and the red sphere, lacking any nucleus, does not develop (Photographs 10, 11). If the sperm is in the red sphere, both the half eggs may develop, one (the white) having the female nucleus only, the other (the red) the male nucleus (Photograph 12). The white sphere, however, sometimes does not develop when the red sphere does; in this case presumably artificial parthenogenesis is not initiated in the white sphere by the sperm before its segregation in the red sphere.

PARTHENOGENETIC EGGS

Having found that fertilized and unfertilized eggs react quite differently to centrifugal force, observations were made on parthenogenetic eggs. Unfertilized eggs were started to develop parthenogenetically by treating them for a few seconds with distilled water; by this method, they develop only up to first cleavage. When placed immediately after treatment in the centrifuge slide, they broke apart very readily at high speed. With low speed, acting while the (fertilization) membrane was being raised, the membrane was observed to be stretched and the egg broke into two spheres within the membrane; these two spheres sometimes remained separate and sometimes coalesced on retraction of the membrane when removed from the centrifuge. If the membranes were removed by violent shaking as soon as formed, the parthenogenetic eggs behaved exactly like the fertilized eggs, forming long streamers from five to twenty minutes after treatment, and then becoming more resistant to elongation and breaking. This could be overcome by keeping the eggs previously in calcium-free sea water. Parthenogenetic eggs act, therefore, in every way and at every stage up to the first cleavage exactly like fertilized eggs.

Cleavage during Rotation

When fertilized eggs are put in the centrifuge-microscope 45 minutes after fertilization and centrifuged continuously, cleavage comes in, even with a centrifugal force of 5000 times gravity, about 20 minutes after the uncentrifuged control. Another set of eggs was rotated at high speed (5000 times gravity) for 15 minutes immediately after fertilization, and then at a lower speed (about 2500 times gravity) for an hour, when cleavage took place while rotating (Photograph 8). In all cases observed, the cleavage plane came through the oil cap and divided the egg along its long axis. It is an interesting fact that an egg can pass through all the complicated processes connected with fertilization and cleavage while being rotated at high speed (5000 times a minute).

Summary

1. *Arbacia* eggs centrifuged immediately (30–90 seconds) after fertilization become more elongate dumb-bells and break into halves and quarters more readily (most batches) than unfertilized eggs.

2. If centrifuged one and one-half to five minutes after fertilization, they break still more readily into many small pieces.

3. If centrifuged five to twenty minutes after fertilization (fertilization membranes previously removed), they form long streamers which break along their course. After twenty minutes, they elongate and break less and less readily. Treatment with calcium-free sea water makes them break more readily.

4. Some batches of eggs break more readily at every stage after fertilization than unfertilized eggs. Other batches break more readily only up to a certain stage, then less readily, the stage varying with different batches.

5. Fertilized eggs at all stages stratify less readily (that is, are more viscous) than unfertilized. The greater ease of breaking must therefore be due primarily to surface changes.

6. If centrifuged slowly while the fertilization membrane is being formed, this may be stretched and the egg pulled apart inside the membrane. The two parts may develop separately, either one or both according to the location of the sperm; or they may fuse together.

7. Parthenogenetic eggs at all stages up to cleavage act exactly like fertilized eggs.

8. Eggs may cleave even while being rotated at high speed (5000 times gravity); the cleavage plane passes through the oil cap and elongated axis of the cell.

LITERATURE CITED

Chambers, R., 1919. Changes in Protoplasmic Consistency and Their Relation to Cell Division. *Jour. Gen. Physiol.*, **2**: 49.

Cole, K. S., and E. M. Michaelis, 1932. Surface Forces of Fertilized Arbacia Eggs. *Jour. Cell. and Comp. Physiol.*, **2**: 121.

Harvey, E. B., 1932. The Development of Half and Quarter Eggs of Arbacia punctulata and of Strongly Centrifuged Whole Eggs. *Biol. Bull.*, **62**: 155.

Harvey, E. B., 1933. Development of the Parts of Sea Urchin Eggs Separated by Centrifugal Force. *Biol. Bull.*, **64**: 125.

Harvey, E. N., 1933. A New Form of Centrifuge-Microscope for Simultaneous Observation of Control and Experimental Material. *Science*, **77**: 430.

Harvey, E. N., and A. L. Loomis, 1930. A Microscope-Centrifuge. *Science*, **72**: 42.

Heilbrunn, L. V., 1915. Studies in Artificial Parthenogenesis. II. Physical changes in the egg of Arbacia. *Biol. Bull.*, **29**: 149.

Heilbrunn, L. V., 1928. The Colloid Chemistry of Protoplasm. Berlin.

Herbst, C., 1900. Über das Auseinandergehen von Furchungs- und Gewebezellen in kalkfreiem Medium. *Arch. f. Entw.-mech.*, **9**: 424.

ARSENIC IN SEA WATER

NORRIS W. RAKESTRAW AND FRANK B. LUTZ

From the Woods Hole Oceanographic Institution [1]

Interest in the occurrence and quantity of arsenic in sea water centers chiefly around its relation to phosphorus: first, the possibility of its utilization by diatoms or other organisms, in place of or in addition to phosphorus; and second, the possible interference of arsenate in the regular routine determination of phosphate so frequently carried out upon sea water samples.

Arsenic has long been known to occur in the sea. Quantitative determinations by Gautier (1), and others (2), using laborious methods, indicated its presence in amounts of the order of 10–100 milligrams per cubic meter.

Gautier found that in surface water (from near the coast of Brittany) containing 10 mg./m.^{3} of total arsenic, 90 per cent was present in inorganic form. At one deep-sea station he found 25 mg./m.^{3} of As at a depth of 10 meters, 10 mg. at 1335 m., and 80 mg. at 5943 m. He was inclined to attribute the high value at the bottom to volcanic influences and the moderately high value in the upper level to some factor associated with diatom growth. Others, following these limited results, have regularly assumed an increase of arsenic with depth.

Chapman (3), while studying the arsenic content of shell-fish and other marine organisms, made sixteen determinations on sea water samples from near the Nore Lightship. He reports 0.14 to 1.0 parts per million, with an average of 0.33; *i.e.*, more than 300 mg./m.^{3}!

Atkins and Wilson (4), in a study of the methods for the determination of phosphate, silicate, and arsenic, have shown good reason for assuming that while arsenate interferes in the colorimetric estimation of phosphate by producing a similar color, arsenite is without effect; furthermore, that since total As is frequently in larger amounts than the colorimetric method indicates phosphate, most of the arsenic in sea water must be present in some other form, presumably arsenite.

It has been reported, on the basis of culture experiments, that arsenate can substitute for phosphate, to a certain extent, as a requirement for the growth of diatoms, but it is unknown to what extent this actually takes place in the sea.

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[1] Contribution No. 29.

Data on the total arsenic content of sea water have been very scarce, and since no application seems to have been made of the Gutzeit method, the superiority of which over the older Marsh method has been frequently demonstrated, the present authors undertook to apply the more recent procedure to a number of sea water samples.

These were obtained during the summer of 1932 in the course of the regular collecting cruises of the vessels, "Atlantis" and "Asterias," of the Woods Hole Oceanographic Institution, from a number of inshore stations in the Gulf of Maine, to the south of Martha's Vineyard, off the entrance to New York Harbor, etc. In addition, two deep-sea stations are listed in the tables, No. 1349, at 38° 58′ N., 64° 30′ W., and No. 1370, at 35° 55′ N., 72° 46′ W.

TABLE I

Data on Concentration of Arsenic in Sea Water Taken at Different Depths at Various Stations

Gulf of Maine								
Station	Depth	As	Station	Depth	As	Station	Depth	As
	m.	*mg./m.*^{3}		*m.*	*mg./m.*^{3}		*m.*	*mg./m.*^{3}
P-1	0	7	H-8	0	17	1333	0	15
	60	15		10	22		9	15
F-15	0	10		20	12		17	22
	10	17		30	13		26	13
	50	21		50	10		35	16
	75	15		75	15		44	16
	100	10		100	17		52	13
G-12	0	9		160	18		65	16
	10	17	1329	0	9		87	12
	30	11		10	11		186	16
	50	10		20	12	1335	0	9
	75	15		30	10		10	11
	105	6		40	29		20	10
G-11	0	9		50	17		30	10
	10	10		60	14		40	7
	20	14		75	11		50	10
	30	12		100	17		60	10
	50	24		240	20	1337	0	15
	75	13	1331	0	17		10	12
	110	10		10	18		20	10
H-3	0	17		19	20		30	10
	10	16		29	15		40	13
	20	16		39	15		50	10
	30	14		48	11		60	11
	50	16		58	16			
	75	(28)		72	18			
	100	10		97	18			
	115	13		194	12			

TABLE I—*Continued*

N.E. of Cape Cod

Station	Depth	As
	m.	*mg./m.*^{3}
B′-5	10	20
	20	20
	30	25
B′-20	0	18
	20	22
	100	20
	243	25

Off Entrance of N. Y. Harbor

Station	Depth	As
	m.	*mg./m.*^{3}
1297	0	9
	60	22
1298	0	16
	70	26

South of Martha's Vineyard

Station	Depth	As
	m.	*mg./m.*^{3}
A-4	0	10
	10	17
	15	11
A-8	0	14
	10	14
	20	17
	26	8

South of Martha's Vineyard (cont.)

Station	Depth	As
	m.	*mg./m.*^{3}
A-25	0	10
	10	11
	20	21
	30	7
	40	10
	50	10
A-28	0	11
	10	21
	20	14
	30	21
	40	(9)
	50	(27)
	57	10

Long. 38° 58′ N.
Lat. 64° 30′ W.

Station	Depth	As
	m.	*mg./m.*^{3}
1349	0	28
	200	17
	300	12
	400	20
	600	12
	800	16
	1000	17

Long. 38° 58′ N.
Lat. 64° 30′ W.
(cont.)

Station	Depth	As
	m.	*mg./m.*^{3}
1349	1200	18
	1600	20
	2000	15
	2500	16
	3000	20
	4000	15

Long. 35° 55′ N.
Lat. 72° 46′ W.

Station	Depth	As
	m.	*mg./m.*^{3}
1370	0	54
	50	22
	100	21
	200	20
	400	20
	600	22
	800	(31)
	1200	16
	1600	17
	2000	16
	2500	15
	3000	13

The Gutzeit method (5), as now commonly employed in the analysis of foods etc., was used without serious modification, concentrating 100 cc. of the water to small volume before use. The length of the stain on the $HgBr_2$-paper was compared for each sample with those resulting from standard solutions of arsenic. The results, with descriptions of the stations and the depths in meters from which the samples were taken, are given in the tables. Figures indicate milligrams of arsenic per cubic meter of water, *i.e.*, parts per billion.

The results from Station K-2, in Buzzards Bay, represent a series of samples taken successively from the same station over a period of several hours and afford, perhaps, a fair indication of the accuracy of the method. Only one variation of more than 6 mg./m.^{3} is found at corresponding depths, and this range must include not only experimental errors but also any actual variation in the water during the time.

In the case of the shallow stations there seems to be a distinct tendency for an increase in arsenic a short distance below the surface, usually with a decrease again at greater depths. This tendency is far from consistent, however, which is to be expected if it is associated with the variable conditions of the phytoplankton, or diatoms, from one station to another.

The two deep stations present a different problem, with their unusually high surface values, which may, perhaps, be purely accidental. But, more important, there is no regular or significant increase of arsenic with depth. Contrasting this with the case of phosphorus, which, at such a station, would show a ten-fold increase, at least, between the surface and bottom, shows that the two elements are utilized in very different manner.

TABLE II

Data on Concentration of Arsenic at Station K-2 in Buzzards Bay at Different Times of Day

Depth	Time				
	6:25 A.M.	9:31 A.M.	12:37 P.M.	3:43 P.M.	6:49 P.M.
m.	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}
0	15	16	11	13	13
5	15	14	14	16	14
10	10	16	12	15	20
15	13	17	15	11	

Out of 155 determinations made, 140, or 90 per cent, fall between 9 and 22 mg./m.^{3} of As. This small range may, we think, be taken as the normal. Only seven samples were found with more than 25 mg. The average and median of all determinations, irrespective of depth or locality, were each 15 mg./m.^{3}.

The data yield two important general results:

1. Failure to bear out the previous findings of Gautier, who reported a considerable increase in As concentration with depth. On the contrary, although a small amount of the As may be removed from the water at certain stations by diatoms etc., this process is not nearly so important or general as in the case of phosphorus.

2. Corroboration of the assumption of Atkins and Wilson that the greater portion of the As in sea water is present in the form of arsenite.

REFERENCES

(1) Gautier, A., 1903. Arsenic dans les eaux de mer, dans le sel gemme, le sel de cuisine, les eaux minérales, etc. Son dosage dans quelques réactifs usuels. *Compt. rend. Acad. des Sci.*, **137**: 232, 374.

(2) Orton, J. H., 1924. Ministry of Agric. and Fisheries Invest., Ser. 2, **6**: 166.

(3) Chapman, A. C., 1926. On the Presence of Compounds of Arsenic in Marine Crustaceans and Shell Fish. *Analyst*, **51**: 548.

(4) Atkins, W. R. G., and E. G. Wilson, 1926. The Colorimetric Estimation of Minute Amounts of Compounds of Silicon, of Phosphorus, and of Arsenic. *Biochem. Jour.*, **20**: 1223.

Atkins, W. R. G., and E. G. Wilson, 1927. The Phosphorus and Arsenic Compounds of Sea-Water. *Jour. Mar. Biol. Ass'n*, **14**: 609.

(5) Assoc. of Official Agric. Chemists. Methods of Analysis, 1925. Second Edition, p. 171.

The Determination of Arsenic in Food Products. U. S. Dept. of Agric. Publication, 1930.

DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION

GEORGE L. CLARKE

(*From the Woods Hole Oceanographic Institution,*[1] *Woods Hole, Massachusetts, and the Laboratory of General Physiology, Harvard University, Cambridge, Massachusetts*)

The investigations of Russell (1926, 1927, 1931), Kikuchi (1930), Worthington (1931), and Southern and Gardiner (1932) reveal the magnitude and the importance of the diurnal migrations of zoöplankton. The observations indicate that light is probably the most important factor controlling these movements, although it is uncertain in just what way the organisms respond to changes in the intensity of light. There is likewise considerable doubt in regard to the parts played by other factors. The present investigation was undertaken to study in more accurate detail the migrations of plankton organisms —particularly copepods—and to test the theories regarding the actual rôle of light.

In previous investigations the intensity of the light in the water was not measured at the same time that the plankton hauls were made.[2] Usually the submarine irradiation was assumed to be proportional to the light present at the surface as indicated by the time of day and the condition of the weather. Russell (1931) has estimated values for the irradiation at various depths on the basis of measurements made on other occasions (Poole and Atkins, 1929). In the present investigation it was therefore proposed to record the irradiation on deck throughout daylight hours when plankton hauls were in progress and to measure the percentage of surface light penetrating to various depths in the water at suitable periods during the day. The intensity of light to which the organisms are subjected at different levels would thus be precisely known.

To improve the accuracy of the determination of the vertical distribution of the animals, five nets were constructed which could be towed simultaneously at different depths and opened and closed in position. I am indebted to Mr. C. O'D. Iselin of the Woods Hole

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[1] Contribution No. 33.

[2] Russell, in collaboration with Atkins, has recently made such observations, the results of which are as yet unreported (see Atkins and Poole, 1933).

Oceanographic Institution for the main ideas involved in the construction of these nets and for the actual design of the fittings. By using these at intervals during the day and night, we hoped to obtain significant information in regard to the vertical migrations taking place.

The observations on the diurnal migration of plankton reported in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution, at Station 1053 (in August, 1931) and at Stations 1285, 1286, and 1287 (in July, 1932)—all in the Gulf of Maine. The details of the measurement of the penetration of daylight are presented in a separate paper (Clarke, 1933).

Method of Making Hauls

The five closing nets were clamped at intervals on the main dredging cable and towed horizontally through the water, thus sampling the plankton at five different depths in one operation. The original nets were made of silk but these were abandoned because they were too easily injured by washing against the cable—a circumstance unavoidable in the method used. The nets employed in 1932 were made of heavy cotton "scrim" (10 strands/cm.) and proved very satisfactory. They are 3 meters long, 75 cm. in diameter at the mouth, 11 cm. in diameter at the bottom, and provided with a pursing rope 1 meter from the mouth.

The canvas sleeve at the bottom of each net fits snugly over the rim of a light brass bucket (11 cm. in diameter and 17.5 cm. deep), similar to that described by Kemp and Hardy (1929). A brass band is slipped over the canvas and clamped tight by means of a bolt with winged nut. The bucket is thus secured firmly to the net and can be attached or removed quickly. An opening on one side covered with netting of monel metal (40 strands/cm.) allows about two-thirds of the water in the bucket to drain off so that the catch can be poured directly into a 1/2 liter bottle without further straining.

The main dredging cable (1.27 cm. in diameter) to which the five nets are attached, travels from the winch forward along the deck and then outboard to the end of a boom located amidships. The cable is kept as nearly vertical as possible by a stream-lined weight (680 kilograms in air) fastened to the lower end. During this investigation the angle which the cable made with the vertical never averaged more than 23° for each towing period. The work of attaching the nets to the cable is performed at the end of a platform rigged out from the side of the ship.

Each net is laced to its frame, consisting of a brass hoop, and this is attached directly to the cable by means of clamps, no bridle being

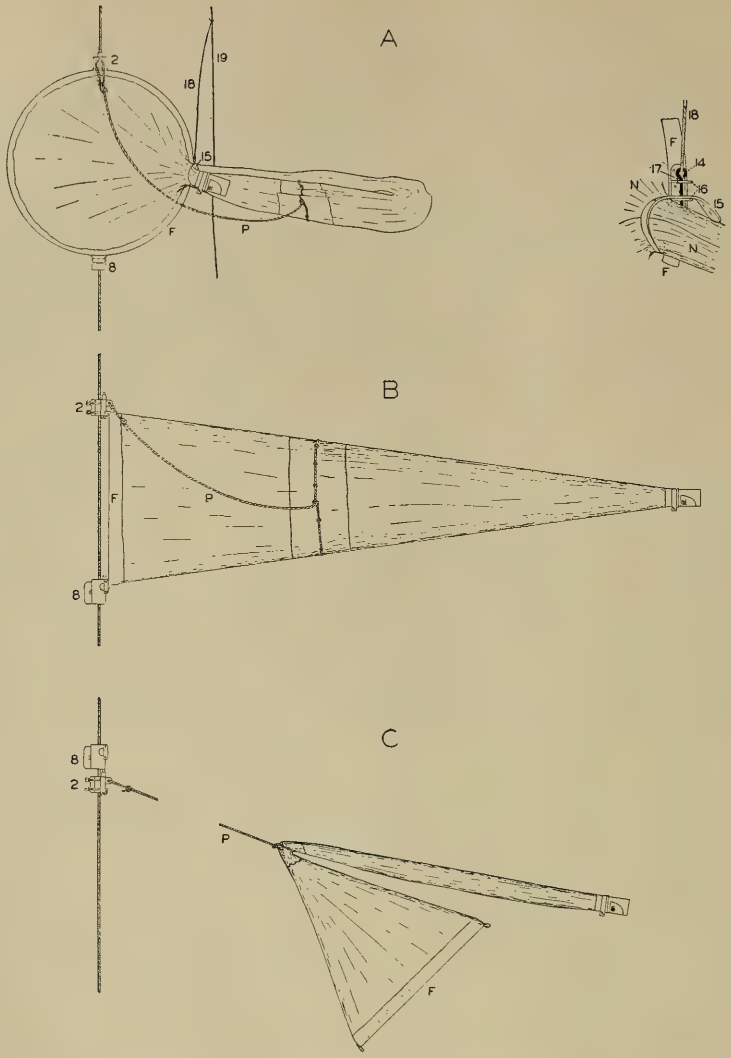

FIG. 1. Diagrams to show the method of using the nets.

A. Net closed and arranged for lowering preparatory to making haul. Net frame moves edgewise through the water.

Inset: Enlargement of edge of net frame showing in detail the strap and pin device.

B. Net open and fishing. The opening is unobstructed except for the single cable passing across it.

C. Net closed and ready to be hoisted aboard. The net frame has been cast off and the net is drawn shut on the pursing rope. Note the "messenger" clamp (8) which has dropped from the net above on to the trigger of the upper clamp (2), thus releasing the closing mechanism.

14. Pin.
15. Strap.
16. Brackets.

F. Frame.

17. Thread stopper.
18. Cord attaching pin to (19).
19. Hand-line to which all the pins are attached.

N. Netting.

P. Pursing rope.

used (see Fig. 1, *B*). The top clamp is composed of two separate parts, as shown in Fig. 2, *A*. The split lug (1), which is cast to fit the lay of the cable, is first rigidly fixed in position by tightening the four screws. Since the lug is small enough to pass through the sheaves, this operation may be performed on deck and the cable then lowered away until the lug reaches a position about two meters above the water. The second part (2), of the upper clamp, is next closed around the lug as shown in *B* (Fig. 2). This fitting swivels freely around the lug. The upper edge of the net frame is provided with a flanged plate (3) which is next secured in the slot (4) of the fitting. This is accomplished by depressing against an internal spring the trigger end (5) of the U-shaped bolt (6). The latch end (7) of the bolt is thus withdrawn from the slot (4), and plate (3) is allowed to slip in. When pressure on the trigger

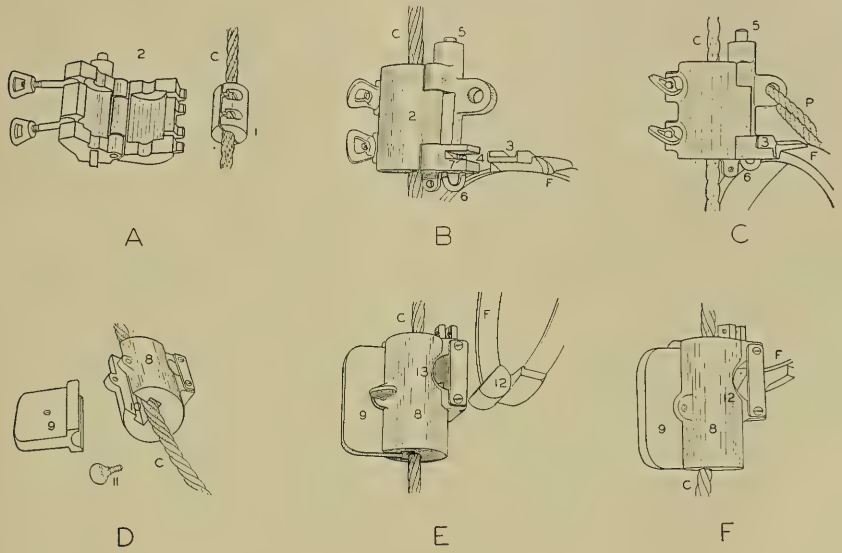

FIG. 2. Method of attaching closing net to cable. For explanation see text.

A, *B*, and *C*—upper clamp and top of net frame.
D, *E*, and *F*—lower clamp and bottom of net frame.

1. Split lug secured to cable.
2. Outside part of upper clamp which encloses (1).
3. Flanged plate at top of frame.
4. Slot into which (3) fits.
5. Trigger end of (6).
6. U-shaped bolt.
7. Latch end of (6).
8. Cylindrical part of lower clamp.
9. Key which slips into (10).
10. Groove in (8).
11. Thumbscrew.
12. Projection at bottom of frame.
13. Socket into which (12) fits.

C. Main dredging cable.
F. Net frame.
P. Pursing rope.

is released, the latch (7) snaps back, passing through a hole in plate (3) and locking it into position (*C*, Fig. 2). The pursing rope (*P*) is made fast to the eye provided in this fitting.

The next step is placing the lower clamp in position as shown in *D* (Fig. 2). The cylindrical part (8) is slipped over the cable and the key (9) is slid up into the groove (10) where it is held by the thumbscrew (11). Before this is done, however, the clamp must be attached to the lower edge of the net frame. This is provided with a projection (12) in the shape of a 3/4-cylinder. The projection can slip into or out of the similarly-shaped socket (13), which is open in front, except when the net frame and the longitudinal axis of the fitting (8) are held parallel. Accordingly, in attaching the lower clamp the fitting (8) is first tilted until the projection (12) slips into the socket (13). It is then straightened up, thus locking the projection in, and the key (9) is slid into place, thus securing clamp and frame to the cable (*F*, Fig. 2). The whole clamp is free to swivel around the cable. Since the upper fitting (2) can also turn, the net frame is free to rotate horizontally although it cannot slide up or down on the cable.

To prevent the net from fishing on the way down (and while the other nets are being attached), it is closed at the mouth by drawing the netting across the opening to one edge of the frame (see Fig. 1, *A*). The netting is held here by a strap, the lower end of which is permanently fastened to the frame. The upper end is provided with a hole (see inset, Fig. 1, *A*) through which the brass pin (14) passes, thus securing the strap (15) between the two brackets (16). A thread stopper (17) prevents the pin from coming out fortuitously. The tail of the net is doubled back and placed under the strap, because if left free, it would lash about and become fouled. The pin (14) is attached to the hand-line (19) by the short piece of cord (18). The pins of all the nets are similarly fastened in succession to this line.

The net is now ready for lowering and the ship is put under way at the lowest possible speed. As soon as the net is in the water, it is swung around by the loose netting trailing out behind. The net frame accordingly tows edgewise through the water and does not fish. Meanwhile the next lug has been attached to the cable at the proper distance inboard. This is run out to the position over the water where the next net is attached exactly as before.

When all the nets have been attached to the cable and the whole string lowered to the desired depth, the ship is accelerated to the regular towing speed (see below). The nets are then opened simultaneously by hauling on the handline (19). When all the pins are pulled free, the line with the five pins attached is taken in on deck. Instantly the pressure of the water causes the nets to open out and to fish as shown in *B*, Fig. 1.

At the end of the towing period the nets are all closed before they are raised to the surface. This is accomplished as follows. A "messenger" (an extra lower clamp) is placed on the cable and allowed to slide down it. When this reaches the topmost net, it strikes the trigger (5). The latch end (7) of the bolt is thereby forced downward and the plate (3) is pulled free by the horizontal pressure of the water on the net. Once the frame of the net tilts backward in this way, the projection (12) slips out of its socket and the frame becomes detached from the lower clamp. Since there is now no vertical support for the lower clamp, it immediately slides down the cable and acts as the "messenger" to release the next net. In quick succession the frames of all five nets are thus cast off from the cable, leaving the nets hanging from the upper clamps by their pursing ropes only. The drag of the net through the water causes these to draw tight and to effectively close the nets as is shown in *C*, Fig. 1. Finally, the nets are raised and taken on deck in the reverse order in which they were sent down. With a little practice this whole operation, including the 10-minute tow, can be completed and the nets made ready to be sent down again in about three-quarters of an hour.

The actual depths at which the five nets were towed at the different stations are given in the tables. In general, the nets were spaced in such a way as to sample the upper 50 meters of water to the best advantage. During the middle of the day, when the plankton was expected to occur at its maximum depth, the series of hauls was repeated at a lower level, the five nets then sampling the water between 50 meters and 100 meters. On one day this was done at other hours as well. The angle which the cable made with the vertical was measured in the usual way at the beginning, middle, and end of each towing period and was found to vary ordinarily between 10° and 20°. In those few cases in which the angle during this period averaged more than 20°, the depths of all the nets were corrected by multiplying by the cosine of the average angle.

The towing period was 10 minutes in all cases. The speed of the ship was carefully determined at least three times during the tow by measuring with a stop-watch the time required for a section of the ship's deck 15.2 meters long to pass a chip of wood tossed into the water. By adjusting the speed of the ship from time to time, the average speed for each tow was kept within the limits of 1.0 to 1.8 knots. At a speed of 1.25 knots, the average for all the hauls, the nets would be drawn through 366 meters of water during the 10-minute period, thus theoretically straining 162 cubic meters. Although the speed of towing varied slightly, it is important to note that it was necessarily the same for all five nets on the line. Differences in the number of animals caught at different depths at the same time, therefore, could not be due to variation in the towing speed.

The hours at which the series of plankton hauls were made are indicated in the tables. At Station 1053 five series were made during the morning. Further observations were unfortunately prevented by bad weather. During the 24-hour period comprising Stations 1285 and 1286, four series were made and each repeated at the lower level. At Station 1287 nineteen series (2 repeated) were made during a continuous 48-hour period. In general the series were made at more frequent intervals of time during the early morning and in the evening because the plankton animals are known to migrate most rapidly during those periods.

METHOD OF ESTIMATING CATCHES

The plankton catches were preserved in 10 per cent formalin and transported to the laboratory where the numbers of the different kinds of organisms were estimated by the following sampling method. Each catch was poured into a large cylindrical battery jar graduated in liters. Water was then added until a suitable dilution was attained, the total volume varying, according to the size of the catch, from 1 liter to 10 liters. The liquid was then thoroughly stirred by an electrically driven propeller. As soon as the plankton organisms became evenly distributed, four samples were taken with a dipper of 29-cc. capacity (consisting of a small porcelain crucible provided with a long handle).

Each sample was poured out upon a ruled counting slide (3.5 × 7.2 cm.) and placed under the binocular microscope. By the use of a mechanical stage the whole sample can be rapidly passed in review. Each individual was examined in turn and its species, sex, and age group recorded. The analysis was limited to those forms which occur in significant numbers. If the first two samples did not vary by more than 10 per cent from their average, no more samples were counted. The samples often gave counts closer than this, indicating that the method used is sufficiently accurate for the purpose (see below). If agreement was not good, the third and, if necessary, the fourth sample was examined. In the case of very large catches it was sometimes necessary to sub-sample the original samples. The same method was used but more samples were counted to insure the same degree of accuracy.

SOURCES OF ERROR

One of the most serious sources of error occurring in investigations of this type is the irregularity in the horizontal distribution (*i.e.*, "swarming") of the plankton. The present method, employing five nets towed simultaneously on a single cable, has certain advantages over previous methods in reducing the magnitude of this error. In the first place, the five hauls of a single series contain no error at all from this source when compared among themselves since they were made at the same time and place. But since the successive series of hauls are not made in exactly the same body of water (due to the drift of the ship), "swarming" would introduce an error for comparisons between the hauls in different series. However, since the nets are towed a considerable distance horizontally, this error is less than would result from the use of a single net hauled vertically (cf. Southern and Gardiner, 1932; and Worthington, 1931). This method is likewise an improvement over those in which a single horizontal net has been towed at different depths in succession. In such cases the individual hauls have been made in different bodies of water, thus introducing the possibility of an error within each series (cf. Russell, 1925).

The use of five nets at once has eliminated another error previously encountered. This was due to the relatively long interval between the times when the first haul and the last haul of a series were made and the consequent possibility that the distribution of the plankton had changed meanwhile. Furthermore, the present method is sufficiently quick to enable complete observations to be repeated at intervals of less than an hour.

In view of the precautions taken, as described above, the errors due to differences in speed and duration of towing (and hence in amount of water strained) and in the depths of the nets are probably negligible. Moreover, it is important to note that the speed and duration are of necessity the same for all the hauls in the same series. The distance between the nets on the cable remained the same, of course, throughout each towing period, and could not have varied by more than a fraction of a meter in different series.

The use of closing nets which are allowed to fish only at their proper depths increases the accuracy of the observations over that possible when open nets are used. However, since the nets are towed horizontally, the layers of water between the nets are not sampled and in the present case there was a considerable body of water beneath the lowest net the population of which was not investigated at all. (Bottom at 256 meters at Stations 1285 and 1286, 146 meters at Station 1287.) Since the investigation was undertaken primarily to test the effect of light, attention was centered on the surface layers. Even at the brightest time of day, light sufficiently intense to be measured did not penetrate to a depth greater than about 50 meters. In the case of the organisms for which we have the most accurate information (*Metridia lucens* adult female), the zone of maximum abundance did not sink below the 54-meter depth at the hour when it would be expected to occur at its lowest for the day (see Figs. 3 and 5). It seems probable that the picture of the vertical distribution of this form would not be materially altered if information on the population of the water layers between the levels of the five nets could have been obtained.

Certain other sources of error common to all similar investigations should be mentioned, the importance of which has long been in question. First of all, there is the possibility that because of clogging the nets might vary in the amount of water actually strained. No satisfactory method has yet been devised to remove this source of error. In the present work the precaution of thoroughly washing out the nets after each haul was always taken. Probably more serious is the possibility that the animals escape the net to varying degrees on different occasions. The speed of towing and the size of the mouth of the net (cf. Gardiner, 1933) are important factors in this connection, but whatever their effects may have been in the present investigation, they were the same for all the hauls. Since our nets have no bridles, the water is stirred up to a minimum extent in front of their openings. Whatever ability the plankton animals may have to see the net and to avoid it to a greater extent in bright light than in weak light or darkness would tend to cause differences in the numbers caught (1) between surface catches made during the day and those made at night and (2) between daytime catches made at the surface and those made at depths where the light is greatly reduced. This question has been thoroughly discussed by Russell (1928), Worthington (1931), and Southern and Gardiner (1932). It is useless to compare day and night totals in the present case (as previously done), since no attempt was made to investigate the bottom layers of water.

Finally, the sources of error in the estimation of the catch must be examined. The method used is comparable to those employed by Russell (1925) and Worthington (1931), which appear to be satisfactory. In an extensive investigation of this matter Gardiner (1931) found that the enumeration error when using a similar method (the Hensen Stempel-pipette) was insufficient to account for the variation of the catch. The error resulting from sampling is undoubtedly large, but in view of the huge variation in numbers of animals caught it is probably *relatively* small, if not negligible.

It seems permissible to conclude that the methods used in this investigation are at least as accurate as those previously employed and that the results may be relied upon. The modifications which have been introduced—particularly the use of five simultaneous closing nets—allow special dependence to be placed upon the differences found among the hauls within a single series.

OBSERVATIONS

General Vertical Distribution

The following three species of copepods comprise the bulk of the catches made: *Metridia lucens* (Boeck), *Centropages typicus* (Krøyer), and *Calanus finmarchicus* (Gunner). In most of the hauls made at Stations 1285 and 1286 and in a few of those at Station 1287, quite large quantities of siphonophore fragments were taken as well. A general view of the numbers of each species of copepod present and of the relative depth at which each lives may be obtained from Fig. 3.

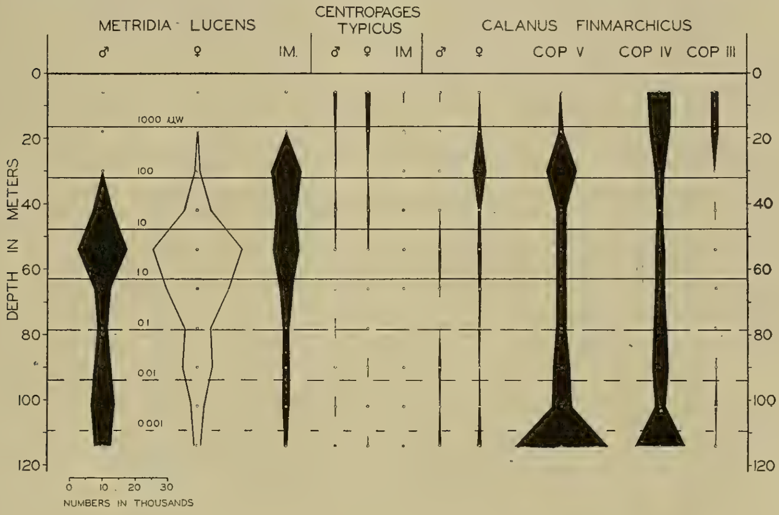

FIG. 3. General view of the vertical distribution and the relative numbers of the three species of copepods studied. The diagram for the adult females of *Metridia* is plotted on one-half the scale of the others. The depth at which light intensities of 1000 microwatts, 100 microwatts, etc., occurred are indicated by the horizontal lines. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) The upper five hauls (A244 to A248) were made at 1439 o'clock, the lower five (Nos. A249 to A253) at 1515 o'clock on July 11, 1932, at Station 1287.

The diagrams, constructed in the usual way (cf. Russell, 1925), represent the copepod population down to 114 meters at Station 1287 early in the afternoon of July 11, 1932. This particular series has been selected as being the most complete and representative. The numerical data for all the series are presented in Tables I, II, and III.

TABLE I

Station 1053, August 24, 1931, Lat. 43°27′N., Long. 67°39′W. Sea rough, sky overcast. Sunrise at 0511. Numbers of copepods in thousands. x = less than 500.

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Imma-ture	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
A55	4.5	0456	19	9.3	No light	282	223	648	0	0	0	0	0
56	14.	"	"	2.7	" "	12	26	4	0	x	x	1	2
57	23.	"	"	1.0	" "	2	9	1	x	11	92	52	27
58	32.	"	"	—	" "	x	2	1	x	15	88	34	3
59	46.	"	"	—	" "	1	2	1	x	6	15	2	0
60	4.5	0602	970	480.	49	16	25	308	0	0	0	0	0
61	14.	"	"	140.	"	103	107	150	0	0	0	0	0
62	23.	"	"	50.	"	1	x	x	x	18	77	53	36
63	32.	"	"	9.1	"	x	x	0	1	13	79	61	11
64	46.	"	"	2.0	"	x	1	x	0	2	11	4	0
65	4.5	0656	1930	950.	57	4	10	153	0	0	0	0	0
66	14.	"	"	270.	"	94	77	69	x	x	x	x	x
67	23.	"	"	100.	"	x	1	x	0	2	26	28	12
68	32.	"	"	18.	"	x	x	x	x	3	32	27	6
69	46.	"	"	4.1	"	x	1	0	x	2	21	13	x

TABLE I—*Continued*

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Immature	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
70	4.5	0807	5000	2450.	64	9	11	296	0	0	0	0	0
71	14.	"	"	700.	"	1	1	35	0	0	0	0	0
72	23.	"	"	260.	"	26	21	84	0	0	0	0	0
73	32.	"	"	47.	"	2	4	1	0	*x*	*x*	*x*	*x*
74	46.	"	"	10.5	"	*x*	*x*	*x*	*x*	*x*	*x*	1	*x*
75	4.5	1227	7100	3500.	150	9	14	360	0	0	*x*	0	0
76	14.	"	"	990.	"	7	12	115	0	0	0	0	0
77	23.	"	"	370.	"	194	220	400	0	0	0	*x*	26
78	32.	"	"	66.	"	2	3	2	0	*x*	11	25	23
79	46.	"	"	15.	"	*x*	1	*x*	0	2	5	5	1
80	64.	1330	7500	3.4	76	0	*x*	*x*	0	2	8	5	*x*
81	83	"	"	—	"	1*	2*	1*	*x*	1	3	1	*x*
82	101	"	"	—	"	*x**	*x**	*x**	*x*	*x*	2	1	*x*
83	120	"	"	—	"	*x**	*x**	1*	0	*x*	2	1	0

* Mostly cast shells.

† 24-hour scale. The time indicated is the middle of the 10-minute towing period.

TABLE II

Hauls A145–A174, Station 1285, July 9, 1932, Lat. 42°34′N., Long. 69°37′W. Sea, slight; sky, clear. Sunrise 0431. Sunset 1939. Hauls A175–A183, Station 1286, July 10, 1932, Lat. 42°30′N., Long. 69°37′W. Sea, slight; sky, few clouds. Sunrise 0431. Sunset 1938.

Numbers of copepods in hundreds. x = less than 50.

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		*μ W*	*μ W*	*per cent*														
A145	5	1119	14,900	6900.	100	x	5	x	0	9	9	9	0	0	x	0	0	0	0
146	15	"	"	1500.	"	0	0	0	0	2	13	6	0	0	2	2	0	0	0
147	25	"	"	330.	"	0	0	0	x	7	15	5	0	0	x	x	0	0	0
148	35	"	"	46.	"	0	0	0	0	2	20	14	x	x	3	10	x	x	0
149	45	"	"	13.	"	0	0	0	0	4	29	19	x	x	1	4	x	1	x
150	55	1230	16,200	3.7	90	0	0	0	x	2	18	19	1	x	3	6	x	1	0
151	65	"	"	—	"	0	0	0	0	3	23	26	1	1	4	14	x	2	0
152	75	"	"	—	"	0	0	0	0	3	43	65	3	1	4	14	x	1	0
153	85	"	"	—	"	0	0	0	0	7	58	84	8	2	7	26	3	6	0
154	95	"	"	—	"	0	0	0	0	3	96.	119	9	3	5	22	0	x	0
155	5	1812	2,700	950.	180	4	15	1	0	2	2	3	0	0	x	0	0	0	0
156	15	"	"	240.	"	1	5	x	0	2	4	2	0	0	x	x	0	0	0
157	25	"	"	110.	"	x	3	0	0	2	10	6	x	x	2	6	0	0	0
158	35	"	"	12.	"	x	1	0	0	x	1	3	x	x	1	4	0	x	0
159	45	"	"	2.7	"	x	x	0	0	x	5	8	x	x	2	5	x	x	0
160	55	1851	1,500	—	"	x	x	0	0	2	35	34	6	7	1	12	0	x	0
161	65	"	"	—	"	0	x	0	0	4	51	58	7	5	x	5	0	x	0
162	75	"	"	—	"	0	0	0	x	3	53	64	6	6	2	6	0	0	0
163	85	"	"	—	"	0	0	0	0	3	36	43	3	1	1	3	0	0	0
164	95	"	"	—	"	2	4	0	0	6	48	37	1	7	3	6	0	0	0

TABLE II—*Continued*

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		*µ W*	*µ W*	*per cent*														
165	5	2123	No light	No light	No light	3	17	1	0	2	5	3	0	2	*x*	3	0	0	0
166	15	"	" "	" "	" "	*x*	2	*x*	0	1	2	*x*	0	1	*x*	4	0	0	0
167	25	"	" "	" "	" "	*x*	0	0	0	3	26	14	1	16	1	8	0	0	0
168	35	"	" "	" "	" "	0	*x*	0	0	3	30	41	2	11	1	6	0	*x*	0
169	45	"	" "	" "	" "	0	*x*	0	*x*	3	29	21	*x*	18	0	6	0	*x*	0
170	55	2214	" "	" "	" "	0	0	0	0	6	42	44	4	72	2	7	0	0	0
171	65	"	" "	" "	" "	0	0	0	0	5	53	58	3	76	3	14	*x*	1	0
172	75	"	" "	" "	" "	0	0	0	0	5	53	68	5	75	5	13	0	*x*	0
173	85	"	" "	" "	" "	0	*x*	0	0	3	40	54	7	58	3	15	0	*x*	0
174	95	"	" "	" "	" "	0	0	0	1	4	40	49	4	76	2	16	*x*	*x*	0
175	5	0552	2,180	1200.	48	8	28	1	0	2	3	3	0	*x*	0	*x*	0	0	0
176	15	"	"	350.	"	*x*	*x*	0	*x*	6	11	7	*x*	*x*	4	8	0	0	0
177	25	"	"	100.	"	*x*	0	0	0	3	21	29	3	1	3	8	*x*	*x*	0
178	35	"	"	21.	"	*x*	*x*	0	0	2	12	25	2	2	2	7	0	*x*	0
179	45	"	"	4.4	"	*x*	*x*	0	0	2	19	43	2	3	1	5	0	*x*	0
180	55	0631	3,950	0.5	63	0	0	0	0	5	41	73	6	1	3	9	0	0	0
181	65	"	"	—	"	0	0	0	0	9	41	85	9	1	2	10	0	0	0
182	75	"	"	—	"	0	0	0	*x*	2	11	19	5	*x*	1	6	0	*x*	0
183	85	"	"	—	"	0	0	0	0	3	24	37	6	1	3	7	0	0	0

TABLE III

Station 1287. Lat. 43°04′N., Long. 69°24′W.

		Sea	Sky	Sunrise	Sunset
Hauls A184–A218	July 10, 1932	Smooth	Clear	0431	1938
Hauls A219–A273	July 11, 1932	Moderate	Overcast, becoming clear	0432	1938
Hauls A274–A293	July 12, 1932	Slight	Clear	0433	1939

Number of copepods in thousands. x = less than 500.

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
A184	6	1418	15,800	7,400.	102	4	11	1	x	1	1	4	1	0	0	0
185	18	"	"	1,100.	"	3	4	0	0	1	1	4	4	0	0	0
186	30	"	"	170.	"	0	x	x	0	2	4	2	x	0	0	x
187	42	"	"	19.	"	0	0	0	x	1	3	2	0	9	50	12
188	54	"	"	—	"	0	0	0	x	1	5	1	0	13	141	25
189	66	1505	13,400	—	108	0	0	0	0	1	4	1	0	3	64	3
190	78	"	"	—	"	x	x	0	0	x	4	2	0	3	10	3
191	(Lost)															
192	102	"	"	—	"	0	0	0	0	0	6	5	x	0	1	2
193	114	"	"	—	"	x	x	x	0	0	11	5	0	x	2	1
199	6	1821	2,050	960.	147	4	7	x	0	1	1	2	2	0	0	x
200	18	"	"	140.	"	x	0	0	0	2	9	5	3	0	0	2
201	30	"	"	22.	"	0	0	0	0	x	4	1	x	0	1	1
202	42	"	"	2.5	"	x	0	0	x	1	2	2	x	8	45	21
203	54	"	"	—	"	0	0	0	x	1	2	1	0	24	89	18
204	6	1854	1,190	560.	173	2	6	0	0	x	1	3	1	0	x	0
205	18	"	"	80.	"	0	0	0	0	2	6	5	2	0	0	1
206	30	"	"	13.	"	0	0	0	0	1	9	3	0	0	4	2
207	42	"	"	1.4	"	0	0	0	0	0	3	2	0	10	78	22
208	54	"	"	—	"	0	0	0	0	1	3	2	x	6	48	13

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
209	6	1952	16	8.	267	5	7	2	0	1	1	11	5	0	*x*	1
210	18	"	"	1.1	"	0	*x*	*x*	*x*	3	2	1	1	0	3	1
211	30	"	"	—	"	0	0	0	0	1	12	2	*x*	0	34	3
212	42	"	"	—	"	0	0	0	0	1	8	3	0	3	91	12
213	54	"	"	—	"	*x*	*x*	0	0	1	2	1	0	13	23	14
214	6	2107	0	0	No light	4	7	1	0	1	1	6	3	0	6	2
215	18	"	"	0	" "	0	0	0	0	7	11	12	4	*x*	46	7
216	30	"	"	0	" "	0	0	0	0	5	21	5	0	0	48	5
217	42	"	"	0	" "	0	0	0	*x*	1	2	1	0	7	12	6
218	54	"	"	0	" "	0	*x*	0	*x*	*x*	4	3	0	12	12	9
219	6	0356	0	0	No light	6	5	1	*x*	2	1	4	1	0	3	1
220	18	"	"	0	" "	0	*x*	0	0	1	5	3	0	*x*	25	6
221	30	"	"	0	" "	0	0	0	0	0	2	1	0	*x*	9	3
222	42	"	"	0	" "	0	0	0	0	1	2	1	0	1	6	4
223	54	"	"	0	" "	*x*	0	0	*x*	1	1	1	0	1	4	3
224	6	0441	114	33.	No light	3	3	2	0	1	1	5	1	0	0	1
225	18	"	"	7.1	" "	*x*	1	0	*x*	2	3	3	*x*	*x*	7	1
226	30	"	"	1.0	" "	0	0	0	0	2	15	3	0	0	32	10
227	42	"	"	—	" "	0	0	0	0	3	8	2	0	2	42	14
228	54	"	"	—	" "	0	0	0	*x*	2	10	2	*x*	6	57	14
229	6	0540	800	230.	24	0	2	1	*x*	1	1	4	1	*x*	1	1
230	18	"	"	50.	"	0	*x*	0	*x*	2	1	2	1	0	7	1
231	30	"	"	7.4	"	0	0	0	0	1	5	3	0	0	12	3
232	42	"	"	1.4	"	0	0	0	0	1	4	1	0	1	17	7
233	54	"	"	—	"	0	*x*	*x*	*x*	1	2	1	0	3	9	5

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
234	6	0742	5,360	1,600.	49	2	2	*x*	*x*	*x*	1	4	2	0	0	0
235	18	"	"	330.	"	0	*x*	0	*x*	4	6	5	1	0	1	2
236	30	"	"	49.	"	0	0	0	0	5	12	5	0	*x*	39	7
237	42	"	"	9.1	"	*x*	0	0	0	3	5	2	0	2	51	7
238	54	"	"	—	"	0	0	0	0	2	5	2	0	2	68	10
239	6	1109	17,100	6,000.	Variable	1	1	0	0	0	*x*	3	2	0	*x*	*x*
240	18	"	"	1,800.	"	1	1	0	*x*	1	1	2	*x*	0	*x*	*x*
241	30	"	"	190.	"	*x*	0	0	*x*	7	33	9	0	0	1	5
242	42	"	"	38.	"	0	*x*	0	*x*	3	7	3	*x*	8	8	12
243	54	"	"	16.	"	0	0	0	*x*	2	5	3	0	6	40	6
244	6	1439	13,900	4,900.	95	1	1	*x*	*x*	*x*	1	7	2	0	0	0
245	18	"	"	1,500.	"	*x*	1	0	0	1	2	6	2	0	*x*	*x*
246	30	"	"	150.	"	0	0	0	0	4	9	3	0	0	3	9
247	42	"	"	31.	"	*x*	*x*	0	0	1	3	2	*x*	6	15	6
248	54	"	"	13.	"	0	0	0	*x*	1	3	3	0	15	55	8
249	66	1515	12,500	0.56	109	0	0	0	*x*	*x*	3	3	0	5	37	4
250	78	"	"	—	"	*x*	0	0	0	*x*	3	4	0	3	15	2
251	90	"	"	—	"	0	*x*	0	*x*	*x*	5	5	*x*	5	19	2
252	102	"	"	—	"	*x*	0	0	0	*x*	6	3	*x*	7	7	2
253	114	"	"	—	"	0	*x*	0	*x*	1	28	16	0	5	3	1
254	6	1821	1,820	660.	180	3	4	*x*	0	*x*	1	5	1	0	0	0
255	18	"	"	130.	"	0	1	0	*x*	3	9	6	1	*x*	0	1
256	30	"	"	9.8	"	0	0	0	0	7	18	2	0	0	98	12
257	42	"	"	2.9	"	0	0	0	*x*	2	9	2	0	*x*	106	18
258	54	"	"	—	"	0	0	0	*x*	*x*	7	*x*	0	7	134	16
259	6	1919	340	120.	300	3	6	1	*x*	1	1	5	2	0	0	0
260	18	"	"	24.	"	*x*	2	0	0	2	2	3	1	0	1	*x*
261	30	"	"	1.8	"	0	0	0	0	*x*	12	0	0	0	153	9
262	42	"	"	—	"	0	0	0	0	0	7	2	0	2	88	14
263	54	"	"	—	"	*x*	*x*	0	0	*x*	3	2	0	7	16	6

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
264	6	2022	0	0	(28 μ W at surface)	5	11	1	0	1	1	5	1	0	1	0
265	18	"	"	0		1	4	0	*x*	1	1	2	1	0	9	1
266	30	"	"	0		0	0	0	0	*x*	5	*x*	0	0	62	2
267	42	"	"	0		*x*	0	0	*x*	3	6	3	0	1	19	4
268	54	"	"	0		0	*x*	0	0	*x*	5	2	*x*	7	17	6
269	6	2126	0	0	No light	5	6	0	0	*x*	1	8	3	0	2	*x*
270	18	"	"	0	" "	1	3	*x*	*x*	1	*x*	2	1	0	2	*x*
271	30	"	"	0	" "	0	0	0	0	3	9	*x*	*x*	*x*	141	21
272	42	"	"	0	" "	0	0	0	1	1	3	2	0	2	28	9
273	54	"	"	0	" "	*x*	0	0	0	*x*	6	4	0	6	18	9
274	6	0337	0	0	No light	2	7	*x*	0	*x*	1	7	2	0	*x*	0
275	18	"	"	0	" "	*x*	*x*	0	0	1	1	3	*x*	0	60	3
276	30	"	"	0	" "	0	0	0	0	2	8	2	0	2	52	3
277	42	"	"	0	" "	0	0	0	0	1	7	3	0	4	21	5
278	54	"	"	0	" "	0	*x*	0	0	3	7	3	0	7	27	6
279	6	0428	124	33.	2	2	6	1	0	1	1	10	2	0	0	0
280	18	"	"	5.8	"	0	1	0	0	1	3	2	1	0	11	2
281	30	"	"	0.6	"	0	0	0	0	*x*	4	1	0	1	38	13
282	42	"	"	—	"	*x*	*x*	0	*x*	2	4	1	0	11	10	4
283	54	"	"	—	"	0	*x*	0	0	2	5	3	*x*	8	22	9
284	6	0542	2,360	640.	47	3	5	1	0	1	1	10	1	0	0	*x*
285	18	"	"	110.	"	*x*	1	0	0	1	2	1	*x*	0	*x*	1
286	30	"	"	12.	"	2	2	*x*	0	1	6	8	2	0	36	11
287	42	"	"	3.8	"	0	0	*x*	0	*x*	2	1	0	13	19	15
288	54	"	"	—	"	0	0	*x*	*x*	*x*	1	1	*x*	11	6	3
289	6	0739	8,930	2,400.	86	1	3	1	*x*	1	1	6	1	0	*x*	0
290	18	"	"	420.	"	*x*	*x*	*x*	0	1	1	1	1	0	0	1
291	30	"	"	46.	"	0	0	0	0	1	6	1	0	0	1	1
292	42	"	"	14.	"	0	0	0	*x*	*x*	2	2	0	0	105	32
293	54	"	"	2.4	"	0	0	0	*x*	0	2	2	0	3	29	7

From Fig. 3 it will be seen that at Station 1287 the adult females of *Metridia lucens* constitute the most numerous group (diagram plotted on half scale) of all the copepods. This group is distributed from 18 meters down to 114 meters, the greatest numbers occurring at the 54-meter level. The depth of maximum abundance of the adult males of this species is also 54 meters, but the range does not extend upwards so far and the animals are relatively more numerous at the lowest depths. All the immature *Metridia* [3] taken together show a maximum at 30 meters, thus exhibiting a tendency to inhabit the upper water layers more commonly than the adults. These observations on *Metridia* agree with Bigelow's conclusions (1924) that the level of plurimum abundance of this species as a whole in the Gulf of Maine is 50 to 150 meters.

Centropages typicus occurs in very much smaller numbers than either *Metridia* or *Calanus*. However, the tendency to concentrate toward the surface is very marked. This is in agreement with previous observations and is undoubtedly correlated with the warmer water found at the surface (Bigelow, 1924, and see below). The maximum numbers were taken at the 6-meter level, for both males and females, although the females were almost equally numerous at a depth of 18 meters. Immature individuals were present at the 6-meter level only.

Calanus finmarchicus is represented chiefly by immature forms, particularly copepodid stage V. The adult males occur in very small numbers at irregular intervals but increasing toward the lower limit of the range investigated (cf. Russell, 1928). The adult females show a maximum at 30 meters. The copepodid stages V and IV are found throughout the vertical range with marked maxima at 114 meters, but individuals of stage IV are relatively more numerous in the surface layers. Copepodid stage III is decidedly concentrated toward the surface. Great irregularities in the vertical distribution of *Calanus* in the Gulf of Maine have been reported previously. The present observations are consistent with Bigelow's conclusion (1924) that this species is far less plentiful in the surface stratum than below 10 meters, at this time of year.

From observations made on the penetration of light into the water at this station (Clarke, 1933), the depths at which intensities of 1000 microwatts, 100 microwatts, etc., occurred have been calculated and these are indicated by horizontal lines at the corresponding depths in

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[3] The numbers of immature individuals in the cases of *Metridia* and of *Centropages* were ordinarily too small to warrant division into age and sex groups. However, this was done for Stations 1285 and 1286 in the case of *Metridia*. I am indebted to Miss Mildred Campbell for working out this further analysis. The immature *Calanus* were divided into age groups for all hauls, following the stages described by Lebour (1916).

Fig. 3 (cf. Russell, 1933).[4] The relationship between the distribution of the copepods and the intensity of irradiation may thus be readily studied. It is seen, for example, that the zone of maximum abundance for adult *Metridia* exists in a region where the intensity of irradiation is between 1 and 10 microwatts. It must be borne in mind, however, that only blue light was measured. If the irradiation over the whole spectrum had been measured, the values obtained would have been greater and the change in light intensity with depth would have been found relatively more rapid, since blue light penetrates the farthest. Moreover, we do not know how this region of the spectrum compares with other regions in stimulating efficiency for copepods. The sensitivity of crustacea in general has been found to extend over a broad range but with a maximum usually in the yellow-green (cf. Bertholf, 1932; Lumer, 1932).

Measurements were made of the lengths of 25 or more individual copepods from each of the groups which were sufficiently numerous. Russell (1928) has shown that measurements of this sort may serve to identify seasonal broods. This information would therefore be useful

TABLE IV

Cephalothorax length of Metridia lucens adult female

Length	Night Hauls			Day Hauls				
	Haul 219 6 m.	Haul 220 18 m.	Haul 223 54 m.	Haul 246 30 m.	Haul 247 42 m.	Haul 248 54 m.	Haul 250 78 m.	Haul 253 114 m.
mm.								
1.743	—	—	—	—	1	—	—	—
1.785	—	—	—	2	2	—	—	—
1.828	—	—	1	—	2	1	—	2
1.870	3	—	—	7	3	5	1	3
1.913	3	2	4	12	13	16	—	5
1.955	2	5	2	10	8	21	3	8
1.998	7	4	4	7	12	24	4	6
2.040	6	5	7	6	4	14	10	3
2.083	1	4	1	2	1	8	7	6
2.125	2	2	6	2	3	3	5	5
2.168	—	2	—	—	—	2	6	5
2.210	1	1	—	1	1	4	6	2
2.253	—	—	—	1	—	2	6	4
2.295	—	—	—	—	—	—	2	—
2.338	—	—	—	—	—	—	—	1
No. measured	25	25	25	50	50	100	50	50
Av. length	2.001	2.037	2.020	1.965	1.958	2.001	2.114	2.046

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[4] For comparing light intensities 1 microwatt may be taken as approximately equal to 3 meter-candles as given by Russell, since his calculations have been based on the data of Poole and Atkins (cf. Clarke, 1933).

for comparison with subsequent investigations. Furthermore, it was desired to inquire whether size varied with depth within each group (cf. Gardiner, 1933). The frequency distributions of the lengths of the copepods are given in Tables IV, V, and VI together with the average lengths. The length of the cephalothorax (including lateral spine, if present) is the dimension considered and not the total length, since the former measurement can be made more quickly and accurately. Both the cephalothorax length and the total length (to the end of the caudal furcæ, but not including the furcal setæ) were measured for 100 individuals of adult female *Metridia lucens* and their ratio was found to be very nearly constant. The average cephalothorax length was 2.001 mm. and the average total length was 3.018 mm., giving an average ratio of 2 : 3 for this group.

From Table IV it may be seen that in the case of the adult females

TABLE V

Cephalothorax length of Metridia lucens and Centropages typicus

Length	Metridia lucens						Centropages typicus	
	Adult Male			Immature			Adult Male	Adult Female
	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 244 6 m.
mm.								
1.063	—	—	—	—	1	—	—	—
1.105	—	—	—	—	—	—	—	—
1.148	1	—	—	—	—	—	—	—
1.190	—	—	—	—	—	—	—	—
1.233	1	—	—	1	—	—	5	—
1.275	8	4	4	—	—	—	4	3
1.318	14	14	6	2	1	—	5	1
1.360	1	3	7	4	2	2	8	—
1.403	—	—	5	2	3	—	2	3
1.455	—	1	3	5	3	—	1	8
1.488	—	3	—	5	10	5	—	3
1.530	—	—	—	3	4	9	—	6
1.573	—	—	—	1	—	3	—	1
1.615	—	—	—	—	—	2	—	—
1.658	—	—	—	—	—	—	—	—
1.700	—	—	—	—	—	—	—	—
1.743	—	—	—	—	—	—	—	—
1.785	—	—	—	1	1	—	—	—
1.828	—	—	—	—	—	—	—	—
1.870	—	—	—	1	—	1	—	—
1.913	—	—	—	—	—	1	—	—
1.955	—	—	—	—	—	2	—	—
No. measured	25	25	25	25	25	25	25	25
Av. length	1.296	1.342	1.356	1.466	1.458	1.583	1.319	1.445

of *Metridia lucens* the individuals in the deeper layers tend to be longer than those nearer the surface although the differences are very small. In the upper layers the average size is slightly greater at night than during the day. If the difference be regarded as significant, it could be interpreted as due to the presence at night of large individuals which had migrated up from the deeper layers. In the case of the adult males and the immature animals of this species there is a similar increase in the number of large specimens at the deeper levels (see Table V). For *Calanus finmarchicus* the data (Table VI) reveal a more pronounced grading effect. Here the difference in the lengths of the age stages may be clearly seen. In addition it will be noted that within each group the frequency distribution definitely moves in the direction of greater length with increasing depth. This observation agrees with that made

TABLE VI

Cephalothorax length of Calanus finmarchicus

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
1.148	—	—	—	—	—	—	—	—	—	1
1.190	—	—	—	—	—	—	—	—	—	5
1.233	—	—	—	—	—	—	—	—	—	5
1.275	—	—	—	—	—	—	—	1	—	6
1.318	—	—	—	—	—	—	—	—	—	5
1.360	—	—	—	—	—	—	—	—	—	3
1.403	—	—	—	—	—	—	—	1	—	—
1.445	—	—	—	—	—	—	—	—	—	—
1.488	—	—	—	—	—	—	—	—	—	—
1.530	—	—	—	—	—	—	1	1	—	—
1.573	—	—	—	—	—	—	5	—	1	—
1.615	—	—	—	—	—	—	7	4	—	—
1.658	—	—	—	—	—	—	5	1	1	—
1.700	—	—	—	—	—	—	4	2	5	—
1.743	—	—	—	—	—	—	1	6	3	—
1.785	—	—	—	—	—	—	2	3	2	—
1.828	—	—	—	—	—	—	—	2	6	—
1.870	—	—	—	—	—	—	—	4	3	—
1.913	—	—	—	—	—	—	—	—	2	—
1.955	—	—	—	4	—	1	—	—	1	—
1.998	—	—	—	3	—	—	—	—	—	—
2.040	—	—	—	2	2	1	—	—	—	—
2.083	—	—	—	2	1	—	—	—	—	—
2.125	1	—	—	2	1	—	—	—	—	—
2.168	2	—	—	1	2	1	—	—	—	—
2.210	1	—	1	3	4	3	—	—	—	—
2.253	2	—	—	3	1	1	—	—	—	—

TABLE VI—*Continued*

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
2.295	3	—	—	1	2	4	—	—	—	—
2.338	—	—	—	—	2	5	—	—	—	—
2.380	2	4	—	1	1	1	—	—	—	—
2.423	2	2	1	3	4	3	—	—	—	—
2.465	—	3	2	—	2	3	—	—	—	—
2.508	2	—	1	—	1	—	—	—	—	—
2.550	2	1	—	—	1	—	—	—	—	—
2.593	1	3	1	—	1	1	—	—	—	—
2.635	4	2	1	—	—	1	—	—	—	—
2.678	1	4	2	—	—	—	—	—	—	—
2.720	1	1	—	—	—	—	—	—	—	—
2.763	—	3	2	—	—	—	—	—	—	—
2.805	1	1	5	—	—	—	—	—	—	—
2.848	—	1	1	—	—	—	—	—	—	—
2.890	—	—	3	—	—	—	—	—	—	—
2.933	—	—	—	—	—	—	—	—	—	—
2.975	—	—	1	—	—	—	—	—	—	—
3.018	—	—	2	—	—	—	—	—	—	—
3.060	—	—	—	—	—	—	—	—	—	—
3.103	—	—	—	—	—	—	—	—	—	—
3.145	—	—	—	—	—	—	—	—	—	—
3.188	—	—	1	—	—	—	—	—	—	—
3.230	—	—	1	—	—	—	—	—	—	—
No. measured	25	25	25	25	25	25	25	25	24	25
Av. length	2.445	2.589	2.766	2.152	2.305	2.328	1.644	1.707	1.787	1.263

by Gardiner (1933) for stage V of this species and indicates that the tendency is of general application for the other stages.

Data on the vertical variations of the following physical and chemical factors were secured by Dr. A. C. Redfield: temperature, salinity, pH, oxygen, nitrate, nitrite, and PO_4. This information is presented graphically in Fig. 4. The temperature—which changes so abruptly between 10 and 20 meters—is probably the most important of these as an agent in determining the vertical distribution of the plankton organisms under consideration (cf. Bigelow, 1924).

It was not possible to obtain measurements of the abundance of the phytoplankton at this station, but observations were made in this vicinity about three weeks later by Gran (1933). He found that the phytoplankton maximum occurred at 20 to 30 meters. The dominating species were *Ceratium fusus*, *Pontosphæra Huxleyi*, and germinating cystæ of *Gonyaulax orientalis*.

Diurnal Migration

In Fig. 5 the changes in the vertical distribution of the adult females of *Metridia* during the 48-hour period comprising Station 1287 are represented diagrammatically. Since this is the most numerous group of copepods, more reliance can be placed upon these observations than upon those of other groups. The first series of hauls made early in the afternoon of July 10 showed that these animals were inhabiting the water from 30 meters down to 54 meters, with maximum abundance at this lower depth. As the afternoon progressed and night came on, their range was extended upwards to the 6-meter level. At the same time the zone of maximum abundance rose gradually. At 21 o'clock the greatest numbers were taken at 30 meters and at 4 o'clock the next morning the maximum occurred at 18 meters. The

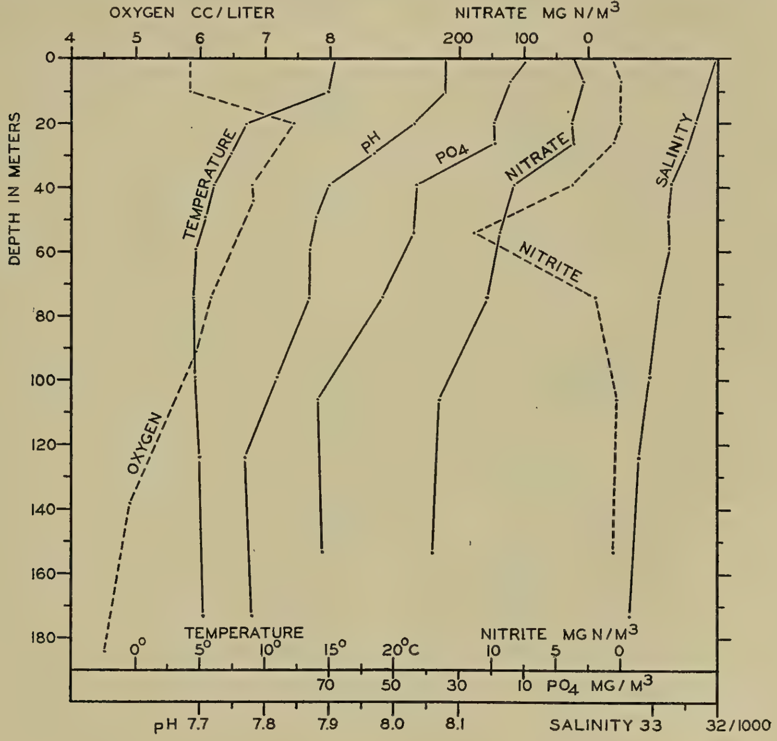

FIG. 4. Variation of physical factors with depth.
Station 1287 (except oxygen which is for Station 1285).

The pH measurements were made by a spectrophotometric method using cresol red as an indicator. The pK value of cresol red was taken as 8.13 and corrected for salinity on the assumption of a salt error of − 0.27. The solutions were measured at a temperature of 20° C. The values are not corrected for the change in temperature.

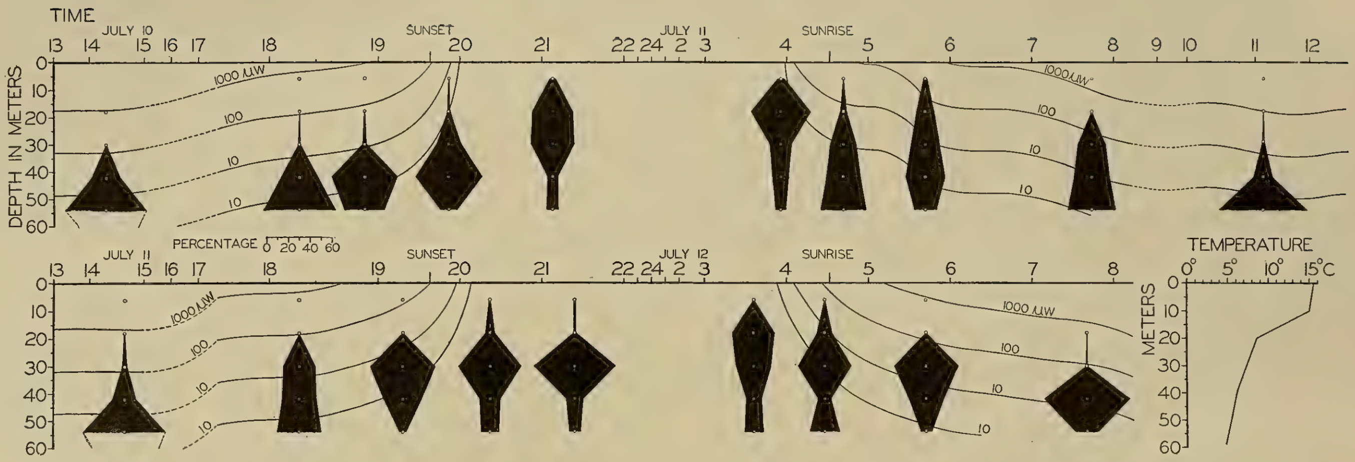

FIG. 5. The diurnal migration of the adult females of *Metridia lucens* during the 48-hour period of Station 1287 (July 10, 11, and 12, 1932). The position of the maximum early each afternoon is shown to be 54 meters, and not lower, by the deep series made immediately afterwards (see Table III). The changes in the light intensity are indicated by the lines representing the depth at which 1000 microwatts, 100 microwatts, etc., occurred. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. The temperature curve for this station is reproduced in the lower right-hand corner.

next series of hauls revealed the beginning of the downward migration. It is seen that the level of maximum abundance fell and the upper layers of water were deserted progressively toward noon. The vertical distribution of female *Metridia* at 0441, with a maximum indicated at 54 meters, does not conform to the gradual change of abundance which is indicated by the other series. The explanation for this is wanting; no corresponding irregularity occurred on the following morning. Early in the afternoon of July 11 the distribution of the animals was found to be very nearly the same as on the previous day. Toward night the animals migrated upward in similar fashion although there are certain differences in the configuration of the population diagrams. As before, the zone of maximum abundance reached the highest level (18 meters) just before dawn. The downward migration ensued as on the previous day, although here again certain differences in distribution do appear.

From the observations of the irradiation on deck and the transparency of the water (tested five times during this station), lines of equal light intensity have been drawn in Fig. 5. An idea can thus be obtained of the actual changes in irradiation to which the organisms were subjected during the course of each day. Inspection of the diagram reveals the fact that in general the zone of maximum abundance of *Metridia* occurs between light intensities of 10 microwatts and 1 microwatt (or less) and rises and falls coincidently throughout the day (cf. Russell, 1927).

At 20 o'clock on July 10 the greatest number of copepods was found below this range of light intensity and at 6 and 8 o'clock on July 12 the maximum was above it. This might be taken to indicate that the animals could not swim fast enough to retain their position within the usual limits of irradiation at those times of day when the light is changing most rapidly. For example, it can be roughly calculated from the figure that the position of the 1-microwatt line rises about 46 meters in the hour between 19 and 20 o'clock on July 10. To keep pace with this a copepod would have to swim vertically upwards at an average speed of 77 cm./min. Parker (1902) found in the laboratory that *Labidocera* swam upwards at the rate of 10 cm./min. The speeds of ascent of the Lucerne plankton observed by Worthington (1931) are even slower than this. However, Welsh (1933) has found that *Centropages typicus* swims toward a light for a distance of 10 cm. in a horizontal trough at average speeds of 71 cm. to 136 cm./min., depending upon the intensity of the light. Such an animal would theoretically be able to swim upwards at a speed equal to this rate minus the rate of sinking due to gravity. Parker observed etherized *Labidocera* to sink at 30 cm./min. and Gardiner (1933) has shown that large specimens of *Calanus* in an anæsthetized condition sink at 50 cm./min. If the sinking rate of *Centropages* is no faster than this, it would appear that this species could ascend at a speed as great as that at which the light changes in the water. Downward swimming at such a rate could probably be easily accomplished. It should be noted that on July 11 no tendency for the zone of maximum abundance of *Metridia* to lag behind the usual range of irradiation is indicated either in the morning or in the evening.

The total range for the population of female *Metridia* never extends above the 1000-microwatt line and it often falls short of this. This value may therefore represent the greatest intensity of light which these organisms can withstand at this period of the year. The copepods do not appear to have been restricted to regions of lower light intensities in the early morning than they were later in the day (cf. Worthington, 1931), thus furnishing no evidence that the organisms became less sensitive to light as the day progressed (cf. Russell, 1931).

The observations consistently indicate that the animals did not tend to become uniformly distributed after complete darkness (cf. Russell, 1926) but continued to migrate toward the surface, reaching their highest average level just before dawn. However, the zone of maximum abundance never rose above 18 meters and very few individuals were taken at the 6-meter level. The stratum of warm water (15° C.) present at the surface, sharply delineated between 10 and 20 meters from the cold water (5° to 8° C.) beneath it (cf. Fig. 5), undoubtedly played a large part in deterring the animals from moving much above the 18-meter level (cf. Russell, 1927; Bigelow, 1924).[5] The downward migration in the morning did not begin before the irradiation in the surface layers had become measurably intense, but soon after this a rapid descent was initiated, for a considerable movement had been accomplished by sunrise.

The behavior of the other groups of copepods is less clearly defined. At Station 1053 (see Table I) *Centropages* was caught in very large numbers in the surface layers. Just before sunrise the great majority of animals in each group was found at a depth of 4.5 meters. As the day progressed, a gradual downward migration took place until noon when the zone of maximum abundance occurred at its greatest

[5] Mr. Russell suggests an alternative interpretation of these observations. He concludes from the diagrams that by dawn the distribution in the deeper layers is tending to uniformity and that if there had been no thermocline the extension of a certain proportion upward would have produced a uniform distribution. The objection to this view is that the animals present at the 30-meter level at 21 o'clock did not spread upwards and downwards in equal numbers during the night but the great majority of them moved up to the 18-meter level.

depth of 23 meters. In each case a number of animals constituting a secondary maximum remained near the surface. The downward movement of *Calanus* during this period was of much smaller proportions. Furthermore, such migration as did occur began later than in the case of *Centropages*. This may represent the difference in time before effective intensities of light reached the deeper levels where the majority of *Calanus* were living. The hydrographic data for this station are as follows:

Depth *meters*	Temp. ° *C.*	Salinity *mille.*
0	16.81	31.97
9	16.82	31.92
17	16.80	31.96
35	16.30	31.96
52	10.60	32.24
69	—	32.31
86	5.86	32.50
104	5.17	32.68

It is evident that the downward migration of *Centropages* was not limited by these factors and that the range of *Calanus* extended right across the thermocline. Very few specimens of *Metridia* were taken at this station.

During the 24-hour period comprising Stations 1285 and 1286 (see Table II) *Centropages* exhibited no vertical migration whatever, maximum numbers being taken in the uppermost net at the four times that observations were made. On the other hand, *Calanus* was taken in every haul at this station. There were extremely few adult males, and the small numbers of adult females taken were distributed irregularly, apparently undergoing no definite migration during the period. However, the groups composed of copepodid stages V and IV respectively showed a fairly regular distribution with a definite maximum in each case. The largest numbers occurred at 95 meters at noon, at 85 meters at dusk, at 65 and 75 meters toward midnight, and at 65 meters the following morning. These forms therefore inhabited slightly greater depths at noon than at other times. The data for the various groups into which the *Metridia* have been divided permit only a similarly general conclusion to be drawn. The zones of maximum abundance for adult males and for adult females existed at 96 meters at noon and at various shallower depths at other periods of the day. The distribution of the copepodid stages is seen to be very irregular.

During the 48-hour period of Station 1287 *Centropages* was found confined to the surface layers as before. In the series made on the first day the range of the adults of this species extended to 18 or 30 meters at noon while the population was chiefly limited to the 6-meter level at other times. This statement does not hold true for the second day, however. The immature group did not leave the uppermost level at any time.

In the case of the adult females of *Calanus* there appears to have been a fairly consistent movement of the zone of maximum abundance from 30 meters at noon, to 6 meters at four o'clock the next morning, and then down to 30 meters again the following noon. After this, however, the maximum occurs irregularly at a variety of depths. Copepodid stage V is represented in every series at all depths. In many cases the population is rather evenly distributed throughout the range. The maximum for each series occurs at the 30-meter level in the majority of cases without regard to time of day. Copepodid stage IV was taken in all but one haul at this station but distribution was irregular. Maxima were found at the 6-meter level for the most part. The prominent maximum found at 114 meters for both stages V and IV may constitute a population which remained at this low level throughout each day and which is distinct from the secondary maximum usually found near the surface on each afternoon. Possibly the animals comprising the latter are those which form the single maximum found regularly in the shallow series made at other periods of the day. The group composed of copepodid stage III was characteristically found in the surface strata. The copepodid stages therefore either failed entirely to migrate, or at most, they moved downwards a short distance during the middle of the day as at the previous station.

The vertical distribution of *Metridia* tends to be more consistent than in the case of *Calanus*. The adult males of *Metridia* are confined to a relatively narrow range. Apparently no diurnal migration was carried out by this group, however, for the maximum in each series except five at this station was found at 54 meters. The pronounced migration of the adult females has already been discussed in detail. The occurrence of the zone of maximum abundance for the immature of this species defies generalization; but it may be concluded that the upper limit of the vertical range of this group exists at a lower depth during the middle of the day than at night.

Discussion

The detailed study of the vertical distribution of these various sex- and age-groups of copepods impresses one with the remarkable differences in behavior exhibited. However, such lack of uniformity among different species, within the same species, and even between different broods, has frequently been reported (*e.g.* Russell, 1928, 1932;

Worthington, 1931; and Southern and Gardiner, 1932). As an example of the differences in behavior of the same species as found by different investigators, our observations on *Calanus finmarchicus* may be compared with those of Russell (1926, 1927). This worker found that the depth of maximum abundance of this species in the Plymouth area changed from about 15 meters at noon to the very surface at night. But according to the data presented above *Calanus* was ordinarily distributed irregularly throughout the upper 50 meters of water and showed only a slight tendency to migrate. Bigelow (1924) has reported considerable lack of conformity in day and night catches of *Calanus* in the Gulf of Maine. In general he finds that ". . . the reactions of *Calanus* in their local application to the gulf result in its being far less plentiful in the surface stratum than below 10 meters or so by day, and often by night, during the half of the year when the temperature is highest and the solar illumination brightest."

Although the nature of the diurnal migration of plankton organisms varies so profoundly, and although different explanations are evidently required for the details of each individual case, still the possibility exists that certain fundamental principles can be found which underlie the phenomenon in its broad outlines. Accordingly, the results of the present investigation will be briefly examined for evidence supporting any of the theories which have been advanced previously.

The absence of vertical movements in the case of some of the animals under circumstances when other forms are migrating is itself a proof that, whatever causal factors may be operating, they are not of uniform application nor effectiveness. Thus, although change of light intensity may have caused the migration undertaken by *Metridia*, it was not equally effective in producing a vertical movement of *Calanus*. Bigelow (1924) says of this species, ". . . its absence on the surface in the regions where it swarms in deeper water is not caused altogether by sunlight, for while it probably does tend to descend during the most brilliantly illuminated hours, on several occasions we have made rich catches on the surface when the sun was high in the sky."

Let us therefore confine our search for a general explanation to those organisms which do migrate, and furthermore to those which move consistently toward the surface at night and downward in the morning. Because of their large numbers and relatively consistent behavior the adult females of *Metridia* form the group from which deduction may most legitimately be drawn in the present investigation. The description of the migration of these animals already presented reveals the fact that the vertical movements definitely coincide with the periods of day and night. Hydrographic measurements made on the preceding days by Dr. A. C. Redfield[6] showed that no significant diurnal changes in the hydrogen-ion concentration, in the oxygen content, or in the temperature were taking place in this region. It seems justifiable to conclude, therefore, as the majority of previous investigators have done, that light is the primary physical factor controlling the vertical migration of these organisms.

The behavior of *Metridia* provides evidence that, in the present case at least, other factors besides light were operating. For, as has already been seen, these animals continue to move toward the surface even after complete darkness and are found at their highest level just before dawn. This type of behavior was found by Worthington (1931) and by Southern and Gardiner (1932) for certain freshwater forms, but is in sharp contrast with observations of Russell (1927) in the Plymouth area, in which many plankton animals were found uniformly distributed in the vertical column of water at midnight. Continued migration after dark may be due to a negative geotropism, as has been suggested by Worthington and others. The possibility of such behavior being correlated with feeding habits (cf. Worthington, 1931; and Southern and Gardiner, 1932) should be examined for *Metridia*. As stated above, the phytoplankton maximum in the present case occurred at a depth of 20 to 30 meters. The upper limit of the vertical movement of *Metridia* corresponds roughly to the upper limit of this zone, but it also corresponds approximately to the beginning of the thermocline (cf. Fig. 5). Pronounced changes in temperature undoubtedly are effective in limiting migrations (cf. Russell, 1927). Furthermore, we have very little information on the actual food of particular species of copepods. More information must be obtained before the importance of feeding reactions in this connection can be decided.

In so far as light is regarded as effective in controlling diurnal migrations, it is worth while to consider in what manner it may exert its influence. The theory that plankton animals tend to keep within a range of "optimum intensity" of light (Russell, 1926, 1927), although supported by a considerable mass of evidence, has failed to fit the facts in certain cases (cf. Worthington, 1931). Question has also been raised as to the acceptability of the "optimum intensity" theory on the basis of experimental work in the laboratory (Clarke, 1930).[7] In the present investigation, however, *Metridia* were found fairly consistently within a definite range of irradiation, as has already been pointed out.

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[6] Unpublished data.

[7] Due to an error, line 26, page 128, of this report became transposed. It should read: ". . . due simply to a negative phototropism and a positive geotropism produced by the rising of the sun in the morning. . . ."

According to other theories, based in large part on laboratory experiments, light is supposed to exert its effect indirectly, by modifying the reactions of the animals to such factors as gravity and temperature (cf. Dice, 1914; Esterly, 1919; Rose, 1925). Whether the control by light is regarded as indirect or as direct, an important distinction should be made between two possible ways in which it may act, viz.: by virtue primarily (1) of its *change* of intensity, or (2) of its *absolute magnitude* of intensity (cf. Clarke, 1930, 1932). The behavior of plankton organisms may be regarded as a series of similar responses to changes in the intensity of light or as different responses to constant intensities of different magnitudes.

Since responses to change of irradiation are ordinarily of short duration, it would be necessary, in order for the first possibility to hold true, for the increase or decrease of light intensity to take place continuously at a rate above the threshold. The requisite rate of change for *Daphnia magna* may be calculated from existing data (Clarke, 1930) and is found never to be less than 16 per cent per minute. In the sea the irradiation changes at a rate which varies according to the time of day but which is ordinarily greatest at sunrise and sunset (cf. Fig. 5). In order to ascertain whether the change of light intensity in the water ever proceeds at a rate above this threshold, a calculation may be made for a time when irradiation is still measurably intense but changing at a maximum speed for that intensity. Between 1900 and 1940 o'clock on July 10 at a depth of 30 meters the irradiation was reduced from 10 microwatts to 1 microwatt. This represents an average rate of change of 4 per cent per minute. If the responses of *Metridia* are to any degree commensurate with those of *Daphnia*, it would appear that the rate of change of light in the sea at no time rose above the threshold value. Nevertheless, an upward movement of the plankton took place during this time. Ewald (1910), recognizing these difficulties, gives reasons for believing that the sensitivity of the animals is increased in nature and that even a gradual change in light intensity might evoke the responses. Judgment on this question must be reserved until more information is available.

On the other hand, if the *change* of irradiation is rejected as a source of stimulation, then the problem resolves into one of the effect of different sensibly constant intensities upon the phototropism, geotropism, etc., of the organisms. In cases, such as *Daphnia*, in which the signs of the tropisms are unchanging under all intensities of light, and in which the animal is able to adapt to light over a wide range, an appeal to these reactions can not account for vertical migration, whether the fundamental feature of it be regarded as a tendency to maintain a position within a certain zone of light intensity or as some other type of behavior. Although these experiments have not yet been proved to give a true picture of the behavior of plankton animals in nature, carefully controlled laboratory investigations of this type have already shed much light upon various aspects of the problem. Whatever theory may be advanced to explain diurnal migration, the underlying reactions involved must be demonstrated conclusively in the laboratory before the explanation can be finally accepted.

SUMMARY

1. Observations on the vertical distribution of copepods and measurements of the submarine irradiation were made in the Gulf of Maine during 12- to 48-hour periods to procure information on the importance of light in controlling diurnal migration.

2. Five closing nets were towed simultaneously at different depths from a single vertical cable. The method devised for allowing the nets to be open only during the 10-minute towing period is described.

3. The sources of error involved in the work at sea and in the sampling of the catch in the laboratory are discussed. The methods used allow special dependence to be placed upon differences found among the hauls within each series.

4. The general vertical distribution of the three species studied was as follows: *Centropages typicus* inhabited the stratum of water above the thermocline (10 to 20 meters), *Calanus finmarchicus* was irregularly distributed, and *Metridia lucens* occurred below the thermocline. In each case the individuals present in the upper part of the range were found to have a slightly smaller average length than those occurring at deeper levels.

5. The adult females of *Metridia* exhibited the most marked diurnal migration, the level of maximum abundance rising in the afternoon and during the night and falling in the morning. These movements were found to coincide to a considerable extent with changes in submarine irradiation.

6. The changes in the vertical distribution of the other groups of copepods were slight or quite irregular. In some cases, however, there was a definite tendency for the maximum to occur at greater depths at noon than at other times.

7. This investigation confirms the idea that light is the most important factor controlling diurnal migration. In addition, the observations are shown to have a bearing on various of the theories regarding the manner in which light exerts its effect.

I am indebted to the Woods Hole Oceanographic Institution for the purchase of the closing nets and for the opportunity to make the observations from the "Atlantis" and to the Laboratory of General Physiology, Harvard University, for facilities for the preparation of the report.

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BIBLIOGRAPHY

ATKINS, W. R. G., AND H. H. POOLE, 1933. The Photo-electric Measurement of the Penetration of Light of Various Wave-lengths into the Sea and the Physiological Bearing of the Results. *Phil. Trans. Roy. Soc. London*, Ser. B, **222**: 129.

BERTHOLF, L. M., 1932. The Extent of the Spectrum for Drosophila and the Distribution of Stimulative Efficiency in It. *Zeitschr. f. vergl. Physiol.*, **18**: 32.

BIGELOW, H. B., 1924. Plankton of the Offshore Waters of the Gulf of Maine. From *Bull. Bur. Fish.*, Vol. 40: Part 2. Document No. 968.

CLARKE, G. L., 1930. Change of Phototropic and Geotropic Signs in Daphnia Induced by Changes of Light Intensity. *Jour. Exper. Biol.*, **7**: 109.

CLARKE, G. L., 1932. Quantitative Aspects of the Change of Phototropic Sign in Daphnia. *Jour. Exper. Biol.*, **9**: 180.

CLARKE, G. L., 1933. Observations on the Penetration of Daylight into Mid-Atlantic and Coastal Waters. *Biol. Bull.*, **65**: 317.

DICE, L. R., 1914. The Factors Determining the Vertical Movements of Daphnia. *Jour. An. Behavior*, **4**: 229.

ESTERLY, C. O., 1919. Reactions of Various Plankton Animals with Reference to Their Diurnal Migrations. *Univ. Cal. Publ. Zoöl., Berkeley*, **19**: 1.

EWALD, W. F., 1910. Über Orientierung, Lokomotion und Lichtreaktionen einigen Cladoceren und deren Bedeutung für die Theorie der Tropismen. *Biol. Centralbl.*, **30**: 1–16, 49–63, 379–384, 385–399.

GARDINER, A. C., 1931. The Validity of Single Vertical Hauls of the International Net in the Study of the Distribution of the Plankton. *Jour. Mar. Biol. Ass'n.*, **17**: 449.

GARDINER, A. C., 1933. Vertical Distribution in Calanus finmarchicus. *Jour. Mar. Biol. Ass'n*, **18**: 575.

GRAN, H. H., 1933. Studies on the Biology and Chemistry of the Gulf of Maine. 2. Distribution of phytoplankton in August, 1932. *Biol. Bull.*, **64**: 159.

KEMP, S., AND A. C. HARDY, 1929. The Ships, their Equipment, and the Methods Used in Research. *Discovery Reports*, Vol. 1, Part II, pp. 141–232.

KIKUCHI, KENZO, 1930. Diurnal Migration of Plankton Crustacea. *Quart. Rev. Biol.*, **5**: 189.

LEBOUR, M. V., 1916. Stages in the Life History of Calanus finmarchicus (Gunnerus), experimentally reared by Mr. L. R. Crawshay in the Plymouth Laboratory. *Jour. Mar. Biol. Ass'n, N. S.*, **11**: 1.

LUMER, H., 1932. The Reactions of Certain Cladocera to Colored Lights of Equal Intensity. *Ohio Jour. Sci.*, **32**: 218.

PARKER, G. H., 1902. The Reactions of Copepods to Various Stimuli, and the Bearing of this on Daily Depth-migrations. *Bull. U. S. Fish Comm.*, 1901, **21**: 103.

POOLE, H. H., AND W. R. G. ATKINS, 1929. Photo-electric Measurements of Submarine Illumination throughout the Year. *Jour. Mar. Biol. Ass'n, N. S.*, **16**: 297.

ROSE, M., 1925. Contribution a l'étude de la biologie du plankton. Le problème des migrations. *Arch. de zool. expér. et gén.*, **64**: 387.

RUSSELL, F. S., 1925. The Vertical Distribution of Marine Macroplankton—an Observation on Diurnal Changes. *Jour. Mar. Biol. Ass'n, N. S.*, **13**: 769.

RUSSELL, F. S., 1926. The Vertical Distribution of Marine Macroplankton. IV. The apparent importance of light intensity as a controlling factor in the behaviour of certain species in the Plymouth area. *Jour. Mar. Biol. Ass'n, N. S.*, **14**: 415.

RUSSELL, F. S., 1927. The Vertical Distribution of Plankton in the Sea. *Biol. Rev.* and *Biol. Proc. Cambridge Philosophical Soc.*, **2**: 213.

RUSSELL, F. S., 1928. The Vertical Distribution of Marine Macroplankton. VII. Observations on the behavior of Calanus finmarchicus. *Jour. Mar. Biol. Ass'n, N. S.*, **15**: 429.

RUSSELL, F. S., 1931. The Vertical Distribution of Marine Macroplankton. X. Notes on the behavior of Sagitta in the Plymouth area. *Jour. Mar. Biol. Ass'n*, **17**: 391.

RUSSELL, F. S., 1932. Behaviour in Invertebrates. *Nature*, **129**: 473.

RUSSELL, F. S., 1933. On the Biology of Sagitta. IV. Observations on the natural history of S. elegans Verrill and S. setosa J. Müller in the Plymouth area. *Jour. Mar. Biol. Ass'n*, **18**: 559.

SOUTHERN, R., AND A. C. GARDINER, 1932. II. The Diurnal Migrations of the Crustacea of the Plankton in Lough Derg. *Proc. Roy. Irish Acad.*, Sect. B, **40**: 121.

WELSH, J. H., 1933. Light Intensity and the Extent of Activity of Locomotor Muscles as Opposed to Cilia. *Biol. Bull.*, **65**: 168.

WORTHINGTON, E. B., 1931. Vertical Movements of Fresh-water Macroplankton. *Int. Rev. ges. Hydrobiol. u. Hydrograph.*, **25**: 394.

THE BUFFER CAPACITY OF SEA WATER[1]

PHILIP H. MITCHELL AND NORRIS W. RAKESTRAW

(From the Arnold Biological Laboratory and the Metcalf Chemical Laboratory of Brown University and the Woods Hole Oceanographic Institution)

The titration of sea water in order to measure its excess of base, or what may be called its buffer capacity for acids, is not only of interest from the standpoint of oceanography but also of practical value in the detection of acid wastes present in small quantities (Thompson and Bonnar, 1931). Several methods have been employed. Wattenberg (1930) has described a method in which an excess of acid is added to the sea water, CO_2-free air is drawn through the boiling mixture until all CO_2 is removed, and then the water is back-titrated with standard barium hydroxide solution, using a mixture of brom cresol green and methyl red as indicator. Greenberg, Moberg, and Allen (1932) use a method of direct titration with standard acid, using methyl orange as indicator. Both of these methods, and others of which they are typical, require the use of rather elaborate precautions and of more or less cumbersome apparatus.

Of recent methods, that of Thompson and Bonnar (1931) possesses certain distinct advantages. It is rapid and employs apparatus easily handled on board ship. It is especially good in that results are practically independent of the CO_2 tension of the sample.

The principle of the method is simple. A sufficient volume of standard HCl is added to a measured portion of the sample to produce an acidity near the middle of the useful range of brom phenol blue. From this the milliequivalents of hydrogen ion per liter remaining unneutralized are calculated. This value subtracted from the quantity of H ion added per liter gives what might be called the apparent buffer capacity of the water. This value, however, must be corrected for the effect of salts upon hydrogen ion activity and upon the dissociation of the indicator. The correction is made by carrying out the same procedure on a sample of carbonate-free, approximately neutralized sea water of the same salinity as the unknown sample. Subtracting the acid required by the carbonate-free water from that required by the sample gives the buffer capacity of the latter.

It seemed to us that if standard values for the amount of acid required to bring carbonate-free sea water of any given salinity to any

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[1] Contribution No. 31 from the Woods Hole Oceanographic Institution.

given pH could be established, the method would be made much more useful, especially since the difficult part of Thompson and Bonnar's method is the preparation and preservation of neutral, carbonate-free water. Our experience has led to the conclusion that such standard values can be obtained. Sea waters from distinctly different regions, whether clear or cloudy (provided they are filtered before use) will yield the same results within the limits of observational error, when rendered truly carbonate-free.

The method of preparing the "neutralized" water has been slightly modified. Concordant and reproducible results can be obtained only when certain precautions are rigidly adhered to. Filtered sea water, sufficient to half fill a large Pyrex boiling flask, is treated with concentrated H_2SO_4, drop by drop, until a test portion gives a color with brom phenol blue approximately equivalent to that of a Clark and Lubs standard tube of pH 3.4. The water is then boiled for about six hours under a Pyrex reflux condenser, while a stream of CO_2-free air is rapidly drawn through it. The reflux condenser is then removed, and while the water is still boiling, carbonate-free NaOH of about 0.07N strength is added in small portions until a sample of the water quickly cooled to room temperature shows a pH of 6.6 with brom thymol blue. The approach to the condition of near neutrality is followed from time to time by titrating a cooled 25-ml. portion of the water with 0.01N NaOH, using brom thymol blue as indicator. As noted by Thompson and Bonnar, the occurrence of an alkaline condition in the water at any time during the approach to neutrality vitiates the preparation. CO_2 will be taken up so rapidly that the water, even though it is acidified as soon as the slight alkaline condition is discovered, will show acid requirements that are too high.

Measurements are made, after the preparation has been cooled to 20° C., upon various known dilutions with distilled water. The following series, for example, was used: 21, 18, 15, 12, 9, 6, and 3 grams per liter of halide, computed as chlorine. Approximate dilutions are prepared and the precise chlorinity later determined by titration with $AgNO_3$. Each of several 100-ml. portions of each dilution is treated with a suitable amount of 0.075N HCl added from a micro-burette permitting a precision of $\pm$ 0.001 ml. Each of the mixtures is then read at 20° C. with brom phenol blue as indicator in a pH bicolorimeter of the Hastings type. The precision of this instrument, provided its optical system is adjusted to give perfectly uniform illumination on both sides, is far beyond the expectations of one accustomed to older pH colorimetric methods. Differences corresponding to less than 0.01 of a pH unit can be recognised and measured by the experienced observer. From the readings, pH values are computed, using 3.98 as the pK_I of brom phenol blue. This "constant" varies, of course, with salinity, but if the same pK_I of the indicator is used throughout, the so-called "salt-error" is automatically eliminated. The value 3.98 is convenient because it is the one established for brom phenol blue at 20° C. in Clark and Lubs' standard buffer solutions. From the pH value and the quantity of acid added are calculated the milliequivalents of H ion neutralized per liter, or the apparent buffer capacity of the carbonate-free water. The several values for each dilution are plotted against the observed pH values, and when this is done for each of the several dilutions, a contour chart involving the three variables, pH, apparent buffer capacity, and chlorinity of the acidified water, is obtained. By interpolation, apparent buffer capacity for any given pH and chlorinity can be read off.

TABLE I

"Apparent" buffer capacity of carbonate-free, approximately neutralized sea water. Milliequivalents of H ion apparently neutralized per liter.

Cl after adding HCl	pH observed after adding HCl											
	4.10	4.05	4.00	3.95	3.90	3.85	3.80	3.75	3.70	3.65	3.60	3.55
gram/l.												
3	*.048*	.055	*.061*	.071	*.080*	.092	*.105*	.121	*.139*	.156	*.179*	.206
4	.058	.065	.075	.084	.095	.107	.123	.139	.158	.177	.203	.232
5	.067	.075	.087	.097	.110	.122	.140	.157	.177	.198	.226	.256
6	*.076*	.085	.098	.108	*.127*	.137	.155	.173	*.196*	.216	.247	.278
7	.083	.093	*.109*	.120	.135	.150	*.171*	.188	.209	.235	*.267*	.299
8	.090	*.101*	.116	*.129*	.146	*.163*	.182	*.204*	.224	*.253*	.285	.319
9	*.096*	.109	.125	.139	*.155*	.174	.194	.215	*.239*	.267	.302	*.339*
10	.103	.116	*.132*	.147	.167	.185	*.205*	.228	.251	.282	*.317*	.354
11	.109	.123	.140	.156	.176	.194	.215	.239	.264	.295	.332	.370
12	*.114*	*.129*	.146	*.162*	*.179*	*.202*	.225	*.250*	*.277*	*.310*	.345	*.383*
13	.120	.135	*.151*	.170	.190	.212	*.236*	.259	.288	.319	*.358*	.398
14	.124	.140	.158	.175	.197	.220	.243	.268	.298	.330	.369	.412
15	*.128*	.145	.164	.181	*.201*	.227	.250	.276	*.307*	.340	.380	*.423*
16	.133	*.150*	.168	*.187*	.210	*.232*	.259	*.283*	.316	*.351*	.390	.435
17	.137	.153	.173	.192	.215	.240	.266	.290	.324	.360	.400	.446
18	*.141*	.157	.178	.196	*.222*	.247	.273	.298	*.333*	.369	.410	*.457*
19	.145	.160	*.184*	.200	.225	.253	*.280*	.304	.340	.378	*.419*	.465
20	*.149*	.164	*.186*	.204	*.229*	.258	*.283*	.309	*.347*	.386	*.429*	*.476*
21	.152	.166	.190	.208	.234	.263	.292	.315	.354	.394	.336	.484

After this method had become standardized six different collections of sea water were used for extended sets of measurements. Their sources were: Woods Hole Harbor, two samples, and one each from Narragansett Bay, Atlantic Ocean near Newport, Atlantic Ocean south of Martha's Vineyard, and Atlantic Ocean near Bermuda. From the resulting six contour charts, the mean value of the apparent buffer capacity was computed for each of 59 points distributed over the ranges of chlorinity and pH values which, when plotted, yield the contour chart shown in Fig. 1. The computed 59 points (in italics), together with others read off from Fig. 1, are given in Table I.

The precision of these values was tested by computation of the probable error of each of the mean values entered upon the contour chart. Of the 59 points 40 had a probable error of the mean not exceeding ± 0.003, 9 of them had a probable error less than ± 0.001 and the general order of the probable error was ± 0.002. In no case did the probable error exceed ± 0.006 and the less consistent results were all in a range of pH values more acid than pH 3.70, a part of the chart not recommended for use in more precise measurements, for reasons to be discussed presently.

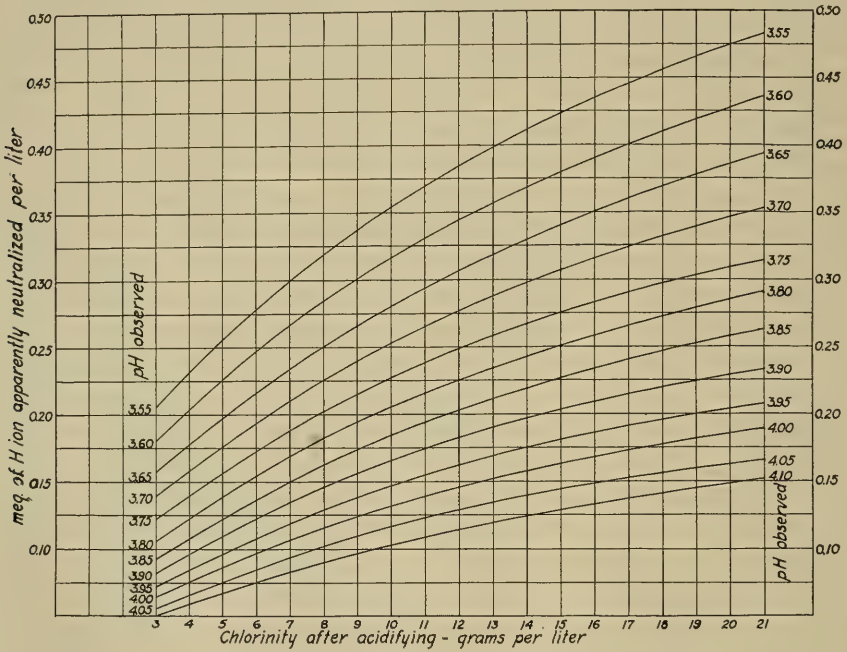

FIG. 1. The apparent buffer capacity of carbonate-free approximately neutralized sea water, used for standard correction values. Above the abscissa equal to the chlorinity of an acidified sea water sample, one selects by interpolation between the isohydric contour lines the pH observed with brom phenol blue as indicator and reads the corresponding ordinate as the correction to be subtracted from the apparent buffer capacity of the sample. These values are computed on the assumption that, for brom phenol blue,

$$pK_t = 3.98 - 0.007(t - 20^\circ \text{ C.}).$$

The final test of the accuracy of these values is their actual use as corrections for measurements of the buffer capacity of sea water. If accurate they should give results on any one sample of sea water, both with and without the addition of distilled water, and at any observed end-point of titration within the chosen pH range, such that the ratio of the buffer capacity (obtained with the use of the carbonate-free correction value) to the chlorinity of the water is constant. This has been found to be the case, in repeated trials, within the limits of observational error. An example of such a test of the method is shown by the results given in Table II.

TABLE II

Titrations of the same sea water sample undiluted and diluted with distilled water

Chloride content	Titration end point	Uncorrected buffer capacity	Correction from contour chart	Corrected buffer capacity	Ratio $\frac{BC}{Cl}$
gram/l.	*pH*	*meq./l.*	*meq./l.*	*meq./l.*	
17.38	3.77	2.394	0.280	2.114	0.1217
17.38	3.79	2.368	0.264	2.104	0.1210
11.98	3.75	1.707	0.247	1.460	0.1219
9.88	3.74	1.439	0.233	1.206	0.1219
5.94	3.87	0.846	0.128	0.718	0.1210
4.05	3.835	0.608	0.115	0.493	0.1217

The selection of an appropriate end-point for the titration of sea water, when brom phenol blue is the indicator, must be a compromise. Greenberg, Moberg and Allen (1932), applying the theory of titration to the problem of methyl orange titration of the total carbonates of sea water, compute the correct end-point as pH 4.35, and use 4.5 in practice. The steepest slope of the titration curve is in the region of pH 4 to 5. This we have found to be the case by the use of the glass electrode described by Taylor and Birnie (1933). This instrument was particularly suitable for the purpose because measurements are taken in a small closed chamber which prevents loss of CO_2. Two titration curves are shown in Fig. 2. They make it obvious that the most sharply defined end-point is at approximately 4.5. Here, however, the effects of carbonic acid are considerable, and loss of CO_2 makes the colorimetric pH estimation very difficult. Indeed, carbonic acid exerts a measurable effect upon the result unless the end-point is distinctly more acid. The true dissociation constant of H_2CO_3, though unknown, is of the general order of pK_a 3.5, according to several observers (Michaelis, 1926). But if the titration is arbitrarily carried to a pH of about 3.4–3.3, that is, beyond the possible effects of carbonic acid, the uncertainty of the colorimetric determination is greatly increased. Readings with brom phenol blue in this range show a comparatively large observational error.

A working compromise between these various objections is reached by selecting the end-point so as to be between pH 3.70 and 4.00, a range within which the titration curve is fairly steep and the measurements can be made with a satisfactory degree of accuracy, while the effects of varying CO_2 tension in the sample are relatively small, affecting the result by an amount less than one per cent of the observed value. The latter fact was ascertained by measuring sea water samples which were equilibrated with laboratory air and then measuring the same samples after bubbling expired air through them. Using 3.98 as the pK_I of brom phenol blue in sea water, as we have proposed above, yields values which are somewhat higher than the *true* pH. In sea water of high salinity at 20° C., the pK_I is of the order of 3.78, as the work of Kolthoff (1930) on KCl solutions has indicated. Precise measurements in sea water of varied salinities and temperatures are desirable. Some preliminary work in this direction has been under-

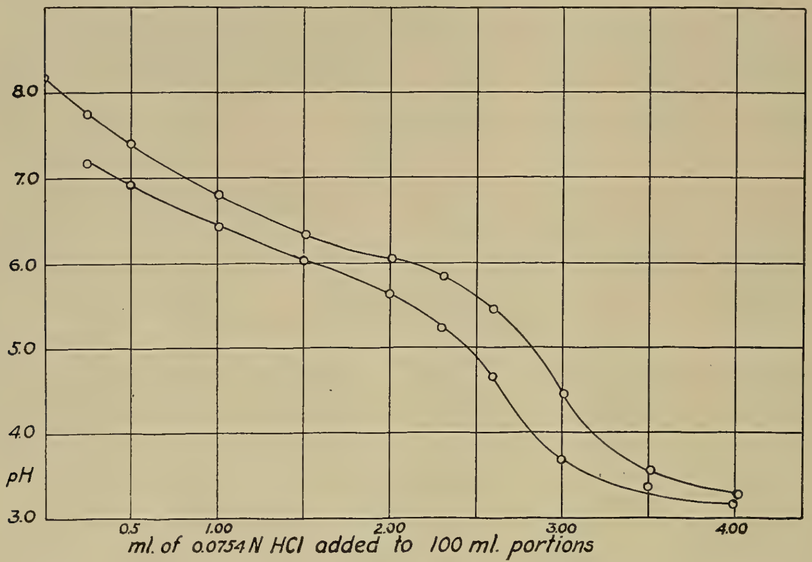

FIG. 2. Sea water titration curves. The pH measurements were taken with a glass electrode in a closed chamber. Each point was obtained with a separate 100-ml. portion of water to which was added the volume of 0.0754N HCl indicated. The pH measurements were made at 30° C.

Upper curve: water from near Newport; chlorinity, 18.10 gram/l.

Lower curve: Narragansett Bay water; chlorinity, 15.21 gram/l.

taken in the laboratory of one of us, using the glass elecrode and the bicolorimeter. It is clear, however, that the end-point chosen for these titrations is sufficiently acid to avoid serious errors due to carbonic acid.

The following procedure is followed in taking the buffer capacity of a sample: It is collected in Pyrex glass, unless measurements can be made at once. Silicates from soda glass can measurably raise the buffer capacity. If it must await analysis for any length of time, it is preserved at a temperature not above 10° C. Fermentation acids can cause low results, in our experience. If any visible organic matter is present the sample is filtered. Protein and perhaps other organic matter raise the buffer capacity. Measurements are taken at or near 20° C. A 100-ml. portion is pipetted into a small Pyrex flask and HCl of about 0.075N strength is added in amount which, as estimated roughly from the salinity of the water, will bring it to the desired acidity as described above. Either preliminary trials or previous experience with water of the locality is necessary to determine the amount. The flask is stoppered and its contents thoroughly mixed and used for pH measurements in the bicolorimeter. The brom phenol blue can give reliable results only when of high purity, which can be ascertained by using it with Clark and Lubs' standard phthalate buffer solutions. Of five different manufacturers' products tested, only two were found to be within the limits of the accuracy of this method. For all the results reported here, brom phenol blue from Hynsonn, Westcott and Dunning was used.

The solution of the indicator must also be approximately isohydric with the solutions to be measured. This condition was sufficiently approximated by adding to the 0.04 per cent indicator solution, as commonly prepared with one molecular equivalent of NaOH, a further amount of 0.01N NaOH equal to one-third of a molecular equivalent. This solution gave the same pH readings in the sea water irrespective of the concentration of indicator. Indicator solutions containing either one or one and one-half molecular equivalents of NaOH failed to do so. An indicator solution containing more than one molecular equivalent of alkali is relatively unstable and should be prepared not more than three days before use.

It is not always feasible to secure temperature control at 20° C. during the pH observation. Although slight variations in temperature do not greatly alter the pH of the acidified sea water, they do affect the dissociation of the indicator to an extent exceeding the limits of accuracy otherwise attainable in this method. As an approximate correction for the temperature effect upon brom phenol blue, we have used the formula, $pK_t = 3.98 - 0.007\ (t - 20°)$, where pK_t is the pK_I at temperature, t. This is taken from a few measurements with the use of the glass electrode and is subject to future correction.

The method of computation of results is shown by the following example. To 100 ml. of Woods Hole Harbor water was added 3.400 ml. of 0.07509N HCl, or 2.553 milliequivalents of H ion per liter of water. The resulting pH was 3.83, or 0.153 meq. of H ion in 1034 ml. of mixture. The apparent uncorrected buffer capacity is 2.553 − 0.153 = 2.400 meq./l. Interpolation from the contour chart of Fig. 1 or from Table I shows that at pH 3.83 and chlorinity of 17.8 grams/l (the value for this mixture), the correction is 0.260 meq./l.

TABLE III

Buffer capacity of surface water from the Cape Cod region

Date	Source of sample	Chloride content	Buffer capacity	Specific buffer capacity
		gram/l.	*meq./l.*	*BC/Cl*
12/19/30	Near Woods Hole	18.31	2.204	0.1203
"	" " "	18.34	2.220	0.1210
"	" " "	18.28	2.188	0.1198
"	" " "	18.28	2.176	0.1189
"	" " "	18.27	2.171	0.1188
"	" " "	18.17	2.165	0.1192
"	" " "	18.21	2.188	0.1200
7/27/32	" " "	18.05	2.161	0.1196
"	" " "	18.05	2.138	0.1183
8/30/32	" " "	18.48	2.181	0.1181
"	" " "	18.48	2.162	0.1170
"	" " "	18.48	2.176	0.1177
9/10/32	" " "	18.32	2.165	0.1182
9/12/32	" " "	18.24	2.164	0.1186
"	" " "	18.35	2.159	0.1176
"	" " "	18.24	2.160	0.1182
1/29/33	" " "	17.90	2.112	0.1181
9/16/32	Buzzards Bay	18.48	2.223	0.1203
"	" "	18.27	2.191	0.1200
9/22/32	Cape Cod Bay	18.11	2.183	0.1205
"	" " "	18.02	2.170	0.1203
"	" " "	18.05	2.187	0.1211
9/23/32	East of Cape Cod	18.22	2.187	0.1200
"	" " " "	18.21	2.189	0.1202
"	" " " "	18.20	2.184	0.1200
"	" " " "	18.19	2.184	0.1201
"	" " " "	18.36	2.200	0.1198
"	" " " "	18.35	2.202	0.1200
8/11/32	35 miles south of Martha's Vineyard	18.48	2.223	0.1203

The buffer capacity of the sample is, then, 2.400 − 0.260 = 2.140. The $AgNO_3$ titration of this water sample gave Cl 18.32 grams l, so that the ratio BC/Cl = 0.1168. The latter may be called the "specific buffer capacity."

Results of observations on various samples from the Cape Cod region and from parts of the Atlantic Ocean and the Caribbean Sea included in a recent cruise of the "Atlantis," are summarized in Tables

TABLE IV

Buffer capacity of sea water from the Gulf of Maine

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1705	0	18.10	2.143	0.1184
	50	18.40	2.200	0.1196
	175	18.74	2.210	0.1179
1706	0	18.10	2.144	0.1185
	50	18.42	2.172	0.1179
	150	18.63	2.182	0.1171
1707	0	18.09	2.211	0.1222
	50	18.25	2.186	0.1198
	125	18.56	2.201	0.1186
1709	0	17.47	2.101	0.1203
	31	17.97	2.130	0.1185
1711	0	17.99	2.161	0.1201
	50	18.16	2.167	0.1193
	175	18.86	2.220	0.1177
1713	0	18.22	2.189	0.1201
	50	18.45	2.207	0.1196
	175	19.17	2.254	0.1176
1717	0	18.08	2.180	0.1206
	50	18.34	2.202	0.1201
	200	19.69	2.290	0.1163
1722	0	18.14	2.104	0.1160
	54	18.43	2.196	0.1192
	114	18.90	2.217	0.1173
	0	18.14	2.173	0.1198
	50	18.445	2.181	0.1182
	250	19.66	2.300	0.1170
1723	0	18.765	2.249	0.1199
	70	18.465	2.215	0.1200

III, IV, V, and VI. (The results in Table VI were obtained by Mr. Homer P. Smith, on board the "Atlantis.") Figure 3 shows the location of the principal stations in the investigation.

The specific buffer capacity, or the ratio of buffer capacity to the

TABLE V

Buffer capacity of Atlantic Ocean water

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1462	0	20.90	2.420	0.1158
	0	20.90	2.452	0.1172
	100	20.90	2.450	0.1171
	2000	19.95	2.393	0.1199
	2000	19.95	2.371	0.1188
St. Georges Harbor, Bermuda	0	21.00	2.403	0.1144
1465	0	21.15	2.438	0.1152
	100	20.17	2.363	0.1171
1467	0	21.20	2.373	0.1120
	100	21.20	2.419	0.1140
	4500	20.17	2.378	0.1179
1469	0	21.20	2.402	0.1134
	100	21.15	2.419	0.1143
	600	20.83	2.386	0.1145
	800	20.45	2.380	0.1164
	1200	20.30	2.347	0.1157
	2000	20.22	2.338	0.1156
	3000	20.20	2.346	0.1161
	4000	20.20	2.346	0.1161
1473	0	21.22	2.384	0.1123
	100	21.20	2.374	0.1122
	500	21.07	2.367	0.1123
	1000	20.35	2.328	0.1144
	1600	20.20	2.317	0.1147
	2000	20.20	2.329	0.1153
	2500	20.20	2.329	0.1153
	3000	20.20	2.317	0.1147
1477	0	20.93	2.356	0.1125
	100	21.17	2.385	0.1126
	500	21.03	2.387	0.1135
	1000	20.27	2.319	0.1144
	1400	20.25	2.319	0.1145
	2000	20.25	2.295	0.1133
	3000	20.20	2.295	0.1136

Table VI

Buffer capacity of water from the Caribbean Sea

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1482	100	20.855	2.406	0.1154
	500	20.375	2.367	0.1162
	1000	19.825	2.384	0.1203
	2000	19.845	2.384	0.1201
	3000	19.785	2.335	0.1180
1513	100	20.495	2.296	0.1120
	500	19.98	2.270	0.1136
	1000	19.745	2.245	0.1137
	2000	19.81	2.271	0.1146
	3000	19.81	2.271	0.1146
1527	0	20.65	2.355	0.1140
	100	20.755	2.389	0.1151
	500	19.77	2.310	0.1168
	1000	19.785	2.322	0.1174
	2000	19.82	2.335	0.1178
	3000	19.83	2.347	0.1184
1548	0	20.35	2.319	0.1140
	100	20.48	2.355	0.1150
	500	19.95	2.310	0.1158
	1000	19.74	2.298	0.1164
1581	0	20.425	2.355	0.1153
	100	20.435	2.368	0.1159
	500	20.07	2.309	0.1150
	1000	19.685	2.311	0.1174
	2000	19.755	2.298	0.1163
	3000	19.775	2.298	0.1162
	4000	19.76	2.258	0.1143
	5000	19.775	2.258	0.1142
	6000	19.775	2.229	0.1127
1598	0	20.38	2.391	0.1173
	100	20.36	2.381	0.1169
	500	20.00	2.346	0.1173
	1000	19.725	2.323	0.1178
	2000	19.77	2.310	0.1168
	3000	19.775	2.327	0.1177
			2.310	0.1168
1606	0	20.415	2.367	0.1159
	100	20.40	2.344	0.1149
	500	20.07	2.296	0.1144

chlorinity, is in general lower than that reported by Thompson and Bonnar for Pacific water of the San Juan Archipelago and nearby regions. That this difference is not due merely to variations in method is indicated by the results of measurements made by one of us on 48 samples from various bays in the Puget Sound region. These measurements were made by the same method as that used in this report and the same correction values for salt effects were applied. All the re-

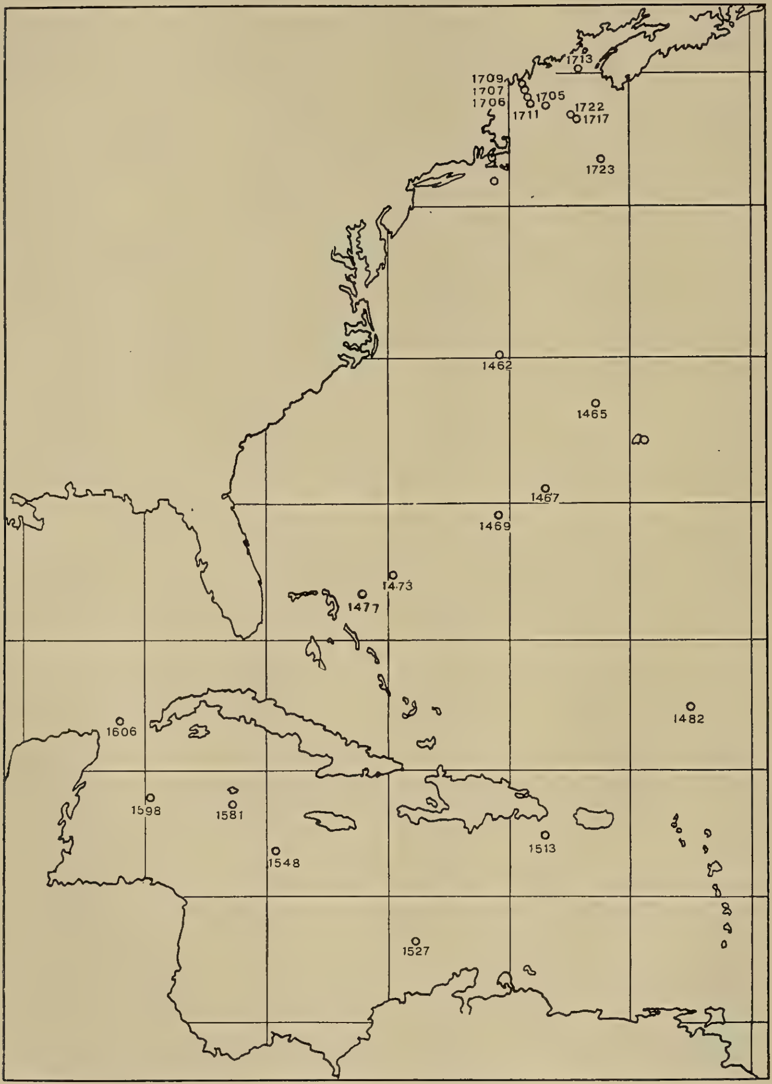
1713
1709
1707
1706
1705
1711
1722
1717
1723
1462
1465
1467
1469
1473
1477
1482
1606
1598
1581
1548
1513
1527

FIG. 3. Location of the principal stations.

sults fell within the range of specific buffer capacities reported by Thompson and Bonnar. It is hoped that the titratable alkalinity of Atlantic and Pacific waters can be more adequately compared in the near future. It would also be of interest to compare the ratios we have already found with those obtained in other parts of the world, but it is not yet certain in our opinion that results obtained by different methods are fairly comparable. So far as we are aware, the report of Thompson and Bonnar is the only one based upon the use of this method.

A comparison of our data with those of Wattenberg (1925–27), in the course of his extensive work in the South Atlantic on the "Meteor" expedition, shows very fair agreement. His values for the ratio of alkalinity to chloride fall mostly between 0.120 and 0.125, but using chloride per kilogram as a basis. When this is changed to chloride per liter (lowering the ratio about 2 per cent) very close agreement results with our average value of 0.119 + for water near Cape Cod.

Two general tendencies are to be seen in the above data: first, a lower specific buffer capacity in waters to the south than in those from the neighborhood of Cape Cod and the Gulf of Maine. This difference is especially noticeable in the western part of the Sargasso Sea and in the greater portion of the Caribbean. A full discussion of the significance of these differences must await further data, which we are endeavoring to obtain.

Were these variations due simply to a difference between coastal and mid-ocean water we should expect the results in the Caribbean to be similar to those in the Gulf of Maine, but it is precisely here that the difference is most marked. Wattenberg's values for "specific alkalinity" in the South Atlantic show a similar, but less pronounced, variation with respect to latitude. Averaging his results from stations in the neighborhood of 40° – 50° S. on the one hand, and those from stations 10° – 20° S. on the other, shows an increase of about 2 per cent, at corresponding depths, in the higher latitudes. A similar analysis of our more limited data reveals a difference of 4–5 per cent.

Second, there is a variation in specific buffer capacity with depth; in general, and especially at deep-sea stations, an *increase* with depth. (Wattenberg found the specific alkalinity to behave in the same manner.) In two widely separated areas, however, this variation is reversed. Both in the Gulf of Maine and in the western part of the Caribbean the buffer capacity consistently *decreases* with increasing depth. We are not yet in a position to discuss the full significance of these facts, beyond suggesting that mass movements of the water are concerned, as well as possible variations in the character of the land drainage.

It may be pointed out that the lowering of the specific buffer capacity resulting from the mixing of mid-ocean and coastal water has been apparent in several of our observations. For example, during a prolonged and violent easterly storm in April samples taken at Woods Hole had a higher chlorinity and correspondingly a lower ratio than any we had ever observed there, between 0.1138 and 0.1149.

In addition to the work here reported, many measurements have been made on samples from the Narragansett Bay region. Here, however, the buffer capacities are variable and of a different order from those observed in ocean water. The effects of land drainage, rainfall, tidal and other currents, etc., involved in a nearly land-locked body of water such as Narragansett Bay, are sufficiently complex to deserve special treatment, which will be undertaken in a later report.

SUMMARY

1. The method of Thompson and Bonnar for measuring the buffer capacity of sea water has been modified by the introduction of the use of standard correction values to make allowance for the effects of salts upon hydrogen ion activity and upon the dissociation of brom phenol blue. These values are reported in tabulated and graphic form.
2. Various sources of error in the method are discussed and corresponding refinements of technique suggested.
3. The specific buffer capacity of Atlantic waters according to these measurements is lower than that of Pacific waters, so far as yet investigated.
4. Lower specific buffer capacities were found in the Atlantic Ocean to the southward and in the Caribbean Sea than in the neighborhood of Cape Cod and the Gulf of Maine.
5. In general, an increase in buffer capacity with respect to depth was observed, but in the Gulf of Maine and the western part of the Caribbean this relation was distinctly reversed.
6. The effects of mass movements of ocean water upon the buffer capacity appear to be traceable.

REFERENCES

GREENBERG, D. M., E. G. MOBERG, AND E. C. ALLEN, 1932. Determination of Carbon Dioxide and Titratable Base in Sea Water. *Ind. Eng. Chem.*, Anal. Ed., **4**: 309.

KOLTHOFF, I. M., 1930. Indicator Constants. *Jour. Phys. Chem.*, **34**: 1466.

MICHAELIS, L., 1926. Hydrogen Ion Concentration, p. 34. Translated by Perlzweig. Baltimore.

TAYLOR, I. R., AND J. H. BIRNIE, 1933. A Micro Vessel for Glass Electrode Determinations of Hydrogen-ion Activity of Biological Fluids. *Science*, **78**: 172.

THOMPSON, T. G., AND R. U. BONNAR, 1931. The Buffer Capacity of Sea Water. *Ind. Eng. Chem.*, Anal. Ed., **3**: 393.

WATTENBERG, H., 1925–27. Wissenschaftliche Ergebnisse der Deutschen Atlantischen Expedition auf dem Forschungs- und Vermessungsschiff "Meteor," **8**: 146.

WATTENBERG, H., 1930. *Ann. d. Hydrographie u. Maritimen Meterol.*, **58**: 277.

INHIBITION OF THE STIMULATING EFFECT OF RED LIGHT ON TESTIS ACTIVITY IN STURNUS VULGARIS (STARLING) BY A RESTRICTED DIET[1]

THOMAS HUME BISSONNETTE

(*From the Institute of Animal Nutrition, School of Agriculture, Cambridge, England, and Trinity College, Hartford, Connecticut*)

Introduction

In experiments on *Sturnus vulgaris* between January and March, 1930, and between December and February, 1930–31, in Hartford, Connecticut, to test the effectiveness of red, green, and white lights of equal luminous intensities in stimulating testis activity, a varied diet rich in proteins, fats, vitamins, and mineral salts, as well as carbohydrates, was used. The complete effectiveness of red light at either 2.6 or 1.7 foot candles, respectively, added for 6 hours after nightfall following a period of daylight normal for the time of year, was indicated, with such diets, in inducing spermatogenic activity outside the normal season for this bird (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931).

A further experiment, such as the one to be described here, seemed necessary to test the relations of diet to this reaction. The results of such an experiment in general indicate that such light does not prove equally effective when the diet is restricted to mashed grains, relatively poor in proteins, fats, vitamins, and salts, and quite different from the diets normal for this bird; though similar to those of grain eaters like sparrows and perhaps pigeons at some seasons.

The first set of experiments in January–March, 1930, are completely described elsewhere and will not be repeated here. They furnish further corroborative evidence of the effectiveness of the light exposures in this respect on full diets.

Studies with Adequate Varied Diets

Between December, 1930, and February, 1931, birds of both sexes were fed a diet composed of meat, bread, vegetables (green and otherwise, cooked and raw), bacon, butter, and fruits, from the college dining-hall, ground in a meat-chopper and mixed with bran and wheat and corn meals, to make the mass mealy and not too moist to be picked up by the birds (Bissonnette, 1932*a*, and other papers; Bissonnette and Wadlund, 1931, 1932, 1933). The birds were exposed to light from electric bulbs, filtered through red and green filters, as described, and to unfiltered white light from similar bulbs. The experiments were repeated from January 24 to February 18 with the same bulbs, slightly weakened by use, and therefore of slightly less intensities. They corroborate the findings from the first experiments, though at a somewhat slower rate of activation. In each set of experiments the luminous intensities of the different colored lights were equal as they reached the roosts of the birds, at about 1.7 foot candles at first. Each set of experiments was controlled by birds similarly caged and fed but receiving no light after nightfall.

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[1] Studies on the Sexual Cycle in Birds. X. Aided by grants from the Committee for Research in Problems of Sex and the Grants-in-aid Committee of the National Research Council of the United States, 1930–32, and by laboratory and library facilities of the School of Agriculture and Zoölogy Laboratories, Cambridge, England, and the Marine Biological Laboratory, Woods Hole, Mass., for which the author wishes to express thanks.

Studies on Restricted "Middlings Mash" Diet

During December and January, 1931–32, at Cambridge, England, another experiment was carried out with red and green lights of about 2.66 foot candles intensity and food rations consisting of "middlings mash" only, without the added meats, fats, vegetables, and fruits. The cages were somewhat smaller than those used most recently in Hartford but not smaller than those first used with results consistent with those from the larger cages. The more uniform light control consequent upon the smaller cages probably affected the results of the exposures to light only to make them more uniform (Bissonnette, 1931*a*, *b*).

Three birds, brought into the laboratory on December 18, 1931, and used as controls without added electric light, in an attempt to learn the avenue of reception of the light stimulus by the birds, were subjected to red light of 2.66 foot candles intensity for 6 hours per night from January 1–22, 1932. This consisted of the light from a 200-watt incandescent bulb filtered through an "H. R. Pyrometer Red" filter, from the Corning Glass Company, at 20 inches distance from the roost. The bulb emitted 2660 lumens and the filter transmitted 3.5 per cent of the luminous intensity. As the birds spent most of their time on the roost and at the food tray, set at the same level, this was the most accurate and constant means of controlling the light flux reaching the birds. Slight variations of intensity, due to different positions on the roost and with relation to it and to the light source, could not be eliminated without interfering with the freedom of action of the birds and must be taken into account in considering the results. They undoubtedly led to variations of effect on individual birds.

Two birds in a similar cage received the same intensity of green light filtered through a "Sextant Green" filter from the same company, transmitting 8.75 per cent of the luminous intensity from a 100-watt bulb at the proper distance from the roost. These birds received their green light exposures from December 18 to January 22—35 days.

No birds in either cage died between January 1 and 22, the period of experimental red light treatment. They had evidently acclimated to the confinement and to the other conditions of the experiment by that time.

The three birds from the red-light cage (2 males and 1 female) and the two from the green-light cage (both males) were killed by pressure in the hand as described in previous papers. Their gonads and other reproductive organs were taken, killed, and fixed at once in Bouin's fluid for 24 hours, dehydrated, and cut from paraffin, stained in Heidenhain's iron hematoxylin, and mounted in balsam. Sections were cut 7.5 and 10 microns thick. From these preparations photomicrographs were made at 335 diameters in all cases, for study and final comparison as a supplement to study of the slides. These are reduced in reproduction for the figures in Plate I to about 240 diameters.

After removal of the intestines, feathers, wings, and legs, the bodies of the birds with the other endocrine glands were preserved for further study of those organs.

Figures 1–9 are photomicrographs taken at 335 diameters and reduced in reproduction to about 224 ×.

FIG. 1. Testis section from a mature bird subjected to experimental red light treatment with 1.7 foot candles for 6 hours per night from December 10 till January 2, 1931 (23 days), on a complete mixed diet.

FIG. 2. Testis section from a young bird subject to similar treatment to that of the bird used for Fig. 1.

FIG. 3. Testis section from "control" bird (mature) on similar diet but without experimental red light treatment for the same time as the birds used for Figs. 1 and 2.

FIG. 4. Testis section from a mature bird subjected to red light exposures at 1.7 foot candles for 6 hours per night from January 24 to February 18, 1931 (25 days), on complete mixed diet.

FIG. 5. Testis section from mature bird subject to experimental red light exposures similar to those of the bird supplying section for Fig. 4.

FIG. 6. Testis section from a young bird used as control for birds supplying sections for Figs. 4 and 5, but without red light exposures at night.

FIG. 7. Testis section from a mature bird subjected to red light exposures at 2.66 foot candles for 6 hours per night from January 1 to 22, 1932, on a diet restricted to "middlings mash" only (22 days).

FIG. 8. Testis section from a mature bird subjected to experimental light exposures similar to those of the bird supplying the section for Fig. 7.

FIG. 9. Testis section from a bird subjected to exposures to green light of 2.66 foot candles for 6 hours per night from December 18 to January 22, 1932, on "middlings mash" restricted diet; for comparison with Figs. 7 and 8, from birds subjected to red light of equal intensity from January 1 to 22, 1932, on the same diet.

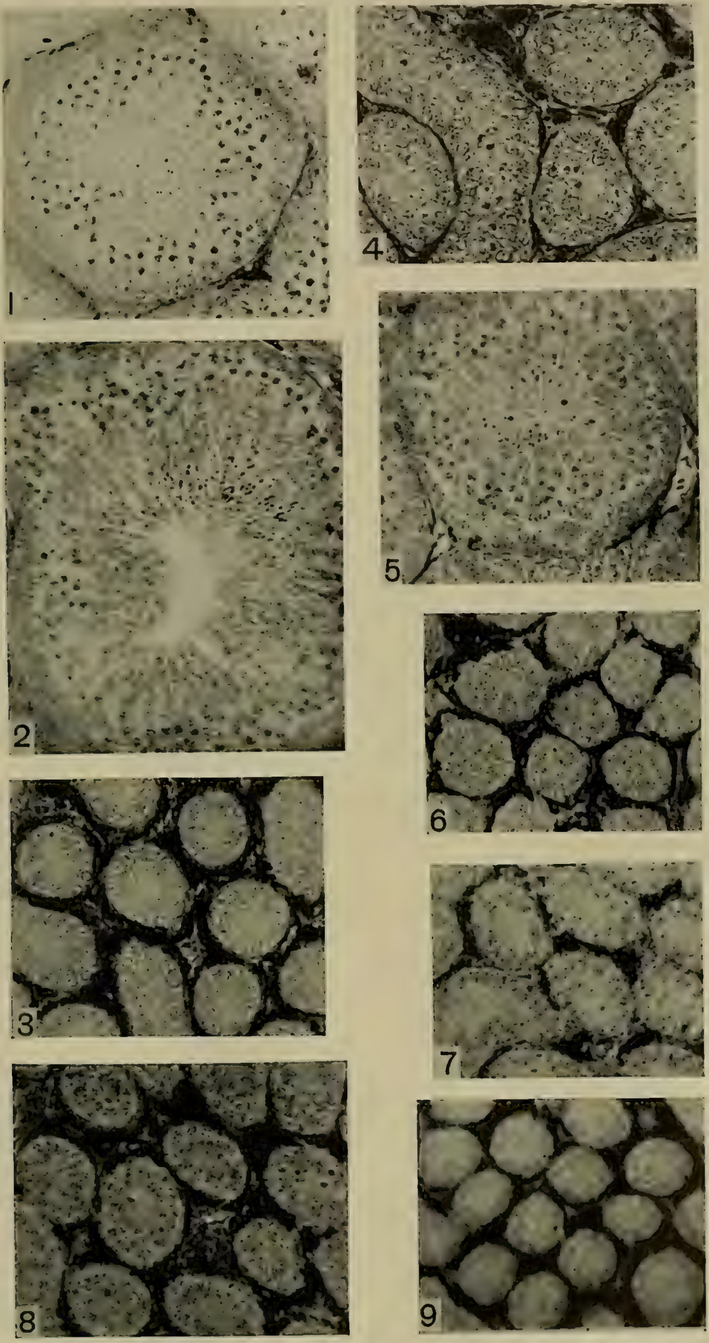

Plate I

Results of Light Exposures on Adequate Varied Diets

After 23 days of 6-hour daily exposures to red light of about 1.7 foot candles (December 10–January 2, 1931), the testis tubules of four birds range from medium size, with early metamorphosing sperms in very small numbers (Fig. 1), to almost maximum size, with much more numerous germ-cells in all advanced stages of maturation, and numerous, almost completely metamorphosed, sperms (Fig. 2).

Control birds, under similar conditions of confinement and food, but without exposures to red light after dark, had testis tubules but slightly above the minimal normal size and without germ-cell stages more advanced than spermatogonia (Fig. 3). Conditions in the tubules of these controls were much more uniform than in the birds exposed to the red light with their variations in numbers of germ-cells of all types. Differences in amount and distribution of pigment and of interstitial cells, as between stimulated birds and controls, were similar to those previously described (Bissonnette, 1930*a*, *b*, 1931*a*, *b*, 1932*a*; Bissonnette and Chapnick, 1930, etc.) and will not be described again here.

After 25 days of similar treatment later in the season (January 24–February 18, 1931), and with intensity slightly smaller from increased use of the same bulbs in the same positions, the conditions in the testes of birds exposed to red light varied from that with the initial appearance of synizesis stages and primary spermatocytes (Fig. 4) to that with medium-sized tubules and metamorphosing sperms (Fig. 5). Controls for the same period, in some cases, had even smaller tubules and fewer germ-cells than those described above (Fig. 6).

Green light of equal luminous intensity and period of exposure induced some reduction of testis size in most cases, with reduced tubule size and fewer spermatogonia, as described in previous papers. They are not reconsidered here (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931, Figs. 28, 36).

Red light of either of these intensities induced complete spermatogenesis in these birds in 23–41 days, depending on the intensity and the relations of the birds to it, in December, January, and February. Testes of birds so treated passed the climax of activity, in spite of increasing periods of light per day, within a time which varied with the time required to induce complete activity. White light of the same intensity was also effective, but at a slower rate. Other experiments with various light combinations, intensities, and daily periods, on similar adequate diets, led to sperm formation in as soon as 18 days after first exposure to the added light, with regression following the climax within 48 days from the first exposures (Bissonnette and Wadlund, 1931, 1932, 1933; Bissonnette, 1932*a*). Green light of like intensities reduced testis size and activity.

These experiments showed conclusively that, on a complete mixed diet, rich in proteins, fats, vitamins, and salts, as well as carbohydrates, sexual activity in these birds is conditioned by daily period, intensity, and wave-length of the light to which they are exposed, and that increase of daily "doses" of visible long-waved light, particularly in the red wave-lengths, induces complete spermatogenic activity even in winter temperatures at the season when testes are normally at their minimum activity or even quiescent. Of the wave-lengths used, and at equal luminous intensity, red light was most stimulating, green was inhibitory, white was less stimulating than red, probably by reason of its smaller content of red wave-lengths (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931). That this was not due to differences in "heat" intensity reaching the birds, but rather to wave-length specificity, was shown by the fact that white light of equal luminous intensity to the red and green, was not intermediate, as were its effects; but was two-fifths the "heat" intensity of the green and one-tenth that of the red; but it was stimulating while green was not. The "heat" intensity was measured with a thermocouple with a variable resistance (Bissonnette and Wadlund, 1931).

RESULTS OF LIGHT EXPOSURES WITH "MIDDLINGS MASH" DIET (Restricted as to Quality but not Quantity)

The testes of the male birds exposed to red light of about 2.66 foot candles for 6 hours per night for 22 days (January 1–22) underwent but little increase in activity and germ-cell maturity beyond that of the controls described above (Figs. 7, 8). Tubules were but slightly larger. In one, germ-cells were less numerous (Fig. 7) than in controls, in the other slightly more numerous (Fig. 8). Necrotic nuclei appeared in both and no germ-cells beyond spermatogonia. Interstitial cells and pigment were like those in controls.

In a female from this cage, the oviduct was enlarged enough to be seen easily with the naked eye, and its walls considerably thickened and modified as compared with controls on normal mixed diet. In the ovary there appeared to be considerable increase in medium-sized follicles but much less change than occurs with similar light exposure on complete diets.

Testes, and the tubules therein, from the two birds exposed to green light of similar intensity on the restricted diet for 35 days (December 18–January 22, 1932) (Fig. 9), were smaller than those of normal birds at the same time of year or than controls (shown in previous papers). Germ-cells were fewer and only a few spermatogonia were present. Pigment and interstitial cells were like those in controls or in winter birds, rather than those of January (Bissonnette and Chapnick, 1930). The necrotic nuclei, found in previous studies during the first 12 days of exposures to green light, had been cleared up by resorption before the thirty-fifth day in this experiment.

Literature and Discussion

The inhibition of the full action of red light upon testis activity, above described, with diet restricted largely to carbohydrates but with increased intensity of light, may be due to one or more of several deficiencies of diet. The experiments here described merely show that such restricted diet is incompatible with the full, or any great, effect on testis activity by increased daily periods of light exposure or of added amounts of long-waved red light. They did not differentiate or distinguish between the possible effects of the several different factors or dietary deficiencies. As shown above, the effect was not entirely eliminated by the dietary deficiencies. There was some acceleration of testis activity as compared with that in controls on an adequate diet but without added light stimulus; but it was relatively slight as compared with effects of even weaker light on the adequate diets. Further studies are planned to analyze this problem. This preliminary report is made lest the impression prevail that our studies indicate that variations of light exposure constitute the only factors conditioning sexual cycles in birds. Results of this work confirm in general the findings of many other studies on birds and mammals, that dietary deficiencies lead to interference with sexual function and rhythm, and with the action of gonadotropic hormones or other stimulators of reproductive activity.

Baker and Ranson (1932, and unpublished data) found that diet for voles may be a major modifying or limiting factor in relation to sexual cycles, either in conjunction with light cycles or without. Similar possible relation of diet to breeding cycles in birds is suggested by Bates (1908), Moreau (1931), and others cited by them, in connection with breeding records of birds in Central Africa, close to the equator, where seasonal changes of day length and of intensity of light are at, or near, a minimum. Many species have two breeding seasons each year related to the dry seasons and so to food; but the situation with individual pairs is not well known. In some species, the sex-glands of males, at least, apparently remain at breeding size and histological condition throughout the year, while nesting and laying are seasonal, possibly in correlation with availability of insects and protein foods as well as with clear skies and changes in quality or quantity of light.

With starlings this sort of relation may obtain, so that lack of certain dietary factors may retard breeding and nesting activity till a high protein diet and certain vitamins are available for feeding the young (such as insects and other live food). But, that changes in food constituents are responsible for sexual cycles in starlings, rather than light cycles, is very unlikely in the light of the studies of Bissonnette and his collaborators, and others, in which food was identical in quality and completeness for both controls and experimental animals subjected to light changes; yet only those exposed to increased lighting of the long-waved sort underwent increased sex-gland activity (Bissonnette, 1930*a*, *b*, 1931*a*, *b*, 1932*a*, *b*, *c*, *d*, 1933; Bissonnette and Chapnick, 1930; Bissonnette and Wadlund, 1931, 1932, 1933; and others cited by them).

Ortiz (1931) reported that experimental beri-beri in cocks did not lead to alteration of sexual conditions or behavior, so lack of antineuritic vitamin may not have played a part in our results.

Byerly, Titus, and Ellis (1933) found that type of protein, at levels between 11.2 and 23.6 per cent in the diet, was a factor in conditioning reproduction and hatchability of eggs in fowl, and that intensity of egg-production, egg weight, and hatchability increased with a rise of percentage of protein in the food, within those limits.

Of the many studies on the relations of various vitamins to reproduction, chiefly on mammals, some are suggestive as indicating possible deficiency factors operating in our starling experiment, and may be considered here.

Sure (1924*a*, *b*, 1926*a*, *b*, 1933), among his studies of dietary requirements for reproduction in rats, found vitamin E necessary for fertility and lactation, when proteins, mineral salts, and vitamins A, B, and D were adequate. This vitamin E he found in wheat embryo among other foods, and it may have been present in the "middlings mash" used in this starling experiment, but hardly in quantity sufficient to permit fully normal sexual reaction. Sure also found vitamin B necessary for both growth and reproduction, and that skimmed milk powder, at 50 per cent level in the ration, yielded enough amino acids of excellent quality for reproduction and with 0.2 per cent of added ferric citrate gave adequate minerals. However, Sure's types of sterility were due largely to resorption of fœtuses rather than to failure of œstrus. His work suggested two fat-soluble factors in wheat oil; one, thermostable and anti-sterile, the other, thermolabile, lactation-promoting. Increase in amount of fats had no beneficial effects on lactation.

Marrian and Parkes (1929) found that female rats rendered anœstrous by vitamin B-deficiency, or by calorific deficiency in their diet, when vitamins were adequate, were immediately stimulated to œstrus and ovulation by the administration of anterior pituitary preparations. So these dietary deficiencies did not prevent gonadotropic action of anterior pituitary hormones in excess of normal. Hill and Parkes (1930) also induced œstrus, copulation, ovulation, corpus luteum formation, with pseudo-pregnancy, in female ferrets during the long winter anœstrous period, by similar preparations. But they report no spermatogenic increase and no enlargement of winter testes in treated males, which, however, copulated with treated females with the above results. Bissonnette (1932*c*, 1933) obtained similar results with ferrets, so far as copulation, œstrus, and pseudo-pregnancies in females are concerned, and some increase in testis size due partly to increase in spermatogenic activity and enlargement of seminiferous tubules and largely to increase of interstitial tissue; but no sperm production, as the result of increased lighting after dark between October and January. The results of Parkes et al., described above, indicate that excess of anterior pituitary hormone can overcome the anœstrum of vitamin B-deficiency and of inanition as well as the seasonal one of winter short days, in some mammals at least. Whether the dose required was greater than for animals not so rendered anœstrous was not determined. The similarity of their results to those of Bissonnette is suggestive of similar cause. In mammals, males are less susceptible to light induction of gonad activity than females, while in birds they appear to be quite as much affected and probably somewhat more so, as has been pointed out elsewhere (Bissonnette, 1932*c*, 1933).

Agnoli (1930) reported that vitamin E-deficiency induced changes in the seminal elements in testes of mice and that these changes could not be prevented by the administration of the lipid hormone from the anterior pituitary. On the ovary no change was induced, but the administration of the hormone led to enlargement of the uterus. Sex-glands of offspring of these vitamin E-deficient mice were modified also. These results are quite in harmony with those in the starling experiment if, as is suspected, the increase of light exposures induces increased anterior pituitary hormone in the blood and so induces sex-gland activation which is inhibited or reduced by restricted diet.

Kudrjaschov (1930) found testis degeneration in vitamin E-deficient rats of various ages with interference with normal spermatogenic function, during periods up to 300 days, leading to loss of spermatogenic cells with loss of size of accessory sex organs, while interstitial tissue was unchanged. He concluded from this that the testis hormone controlling secondary sex-organs originates in the spermatogenic cells rather than in the interstitial cells. The inhibition of germ-cell maturation and multiplication in his rats is, like that induced by the deficient diet in starlings, normally stimulated by the light exposures used. In starlings, however, interstitial cells of the glandular sort appear to be lacking, and the interstitial cells that are present increase in relative amount as the germ-cell elements decrease in volume and activity (Bissonnette, 1930*a*, *b*, 1931*a*, *b*; Bissonnette and Chapnick, 1930).

Vezár (1931) reported that intra-peritoneal injections of vitamin E for over three days into infantile rats induced hypertrophy of the uterus, while subcutaneous injections of like amounts gave positive results in only 20 per cent of the cases, and oral administration led to hypertrophy in three cases. This reaction did not occur in oöphorectomized animals. Vezár and Kokas (1931) found that deficiency of vitamin E for two and one-half months caused the fur in rats to become silky, a condition relieved by administration of anterior pituitary preparations. They therefore conclude that vitamin E has an effect similar to that of anterior pituitary hormones. It seems possible, however, that it may intensify the action of the hormone already being produced while its deficiency may lessen or inhibit it, as probably occurred with the starlings on the restricted diet.

Aberle (1933) found that, in certain states of vitamin A-deficiency in rats, the standardized human placental hormone was prevented from inducing the usual pregnancy cells in the vaginal mucosa—another case of inhibition of the action of a gonadotropic hormone by dietary deficiency.

Mason (1933), in a most interesting study, reported that lack of vitamin C or of vitamin D induced degenerative changes in the testes of guinea pigs and rats which were not prevented by administration of vitamin E; that vitamins A and E are necessary for germ-cell integrity and activity, though their specific effects are different, and different from those of general inanition; that vitamin E-deficiency affects the later stages of germ-cells first, while vitamin A-deficiency permits more activity of germ-cells but depletes their numbers. Repair of rather severe inanition injury can be produced in about three to four weeks of feeding even with diets relatively free of protein. Repair of vitamin A-deficiency injury takes five to thirteen weeks. Mason has distinguished the injurious results of these different dietary deficiencies and finds that vitamin A-deficiency does not act through depletion of anterior pituitary hormone. He suggests that vitamin E is necessary to maintain normal nuclear physiological condition or some phase of chromatin anabolism. If the same situation applies to starlings, deficiencies of one or all of these vitamins would account for failure of the stimulating light to induce much activity in the testes as contrasted with its great effect on complete diets.

Considerable work has been done on the effects of deficiencies of proteins, fats, and minerals upon reproductive capacity. Burr and Sutermeister (1932) found that on given deficient and adequate diets there were no consistent differences in reproduction and ovulation between rats kept in the dark and those given 10 minutes per day of radiation with a carbon arc sunshine lamp. Living in total darkness did not seem to affect reproduction in rats on a simple and highly purified diet. Sorour (1923) found that rats kept in darkness on a ricket-inducing diet developed parathyroid deficiency and a thyroid condition similar to that in Basedow's disease, while those kept in light had normal thyroids. This suggests a control of the thyroid by light through the hypophysis.

Clayton (1930) found that kind or source of protein in the diet was a factor in conditioning reproduction and lactation in rats at a 15 to 20 per cent level, with fats adjusted at 20 per cent level with lard (found detrimental to vitamin E and later omitted), and cod liver oil either given daily or omitted, to avoid its deleterious effect when mixed with the food. Dried egg and kidney were superior to liver and milk proteins and these to muscle protein, probably because of differences of mineral content. As vitamin E-content decreases in the order: egg, milk, round, liver, kidney, the difference could not be due to vitamin E-content alone but to differences in protein. Clayton believes the superiority of egg over other forms of protein is due to its vitamin E-content at the 20 per cent level, more adequately supplemented by minerals and other vitamins. In the raw state, kidney was most adequate for reproduction. Raw egg and raw beef round were poorly assimilated. Vegetable foods proved low in protein and in the fat-soluble vitamin B needed.

Swanson and Nelson (1933), using a 15 to 30 per cent protein diet, found dried pork muscle inadequate for reproduction in albino rats, except in two cases at the 30 per cent level, whereas beef muscle in the same proportions had no bad effect. This indicated that source and type of protein in the diet affect reproductive faculty.

Russell (1932) found that addition of fresh beef or meat scrap to a milk-wheat diet improved reproduction as well as growth rate and vigor of young, in white rats, while dried yeast instead only slightly improved reproduction. He concluded that this benefit of meats could not be chiefly due to increased calcium and phosphorus or to increase of the Ca : P ratio.

Slonaker (1931) found that efficiency of reproduction in rats was at a maximum when proteins were at the 14 per cent level in the diet and fell off in the following order of percentages of proteins: 10, 18, 22, and 26. Sterility in males was greater than in females in each group and sex ratio was below normal in all groups, with a suggested prenatal mortality greater in males than in females, postnatal mortality greater in females. Length of reproductive span, sex ratio of litters, annual number of litters and of young were in the same order related to percentages as above. In size of litter, the 10 per cent group was first, the 14 per cent next, and the others in the same order. In the first three groups males had a longer reproductive span than females; the reverse in the other groups. The period of gestation was, from shortest to longest, in the following order: 10, 14, 26, 22, 18 per cent.

Evans and Bishop (1922*a*, *b*) found length of œstrous cycles closely correlated with state of nutrition of experimental animals; that the 5-day or less œstrous cycle of normal rats on a standard diet was either lengthened or otherwise modified by starvation or by deficiency in proteins, fats, mineral salts, or vitamins; that deficiency of vitamin B or of A gave prolonged diœstrus and cornified stages (2 and 3). Hoffman (1923) confirmed this for rats on an exclusive bread diet, and Wolfe (1930) for mice on a diet deficient in mineral salts. Macy, Outhouse, Long, and Graham (1927) confirmed this for deficiencies of vitamins A and B; but Parkes and Drummond (1926), Coward (1929), and Coward, Morgan, and Dyer (1930) did not.

Lin (1931) and Wu and Chen (1929) found that vegetarian rats grow slower and fail to reach as great a maximum weight as those on omnivorous milk-wheat diet and also suffer from a mild form of nutritional deficiency. Lin, Tsai, and Wan (1932) found that œstrous cycles were not affected by mild nutritional deficiency, but were lengthened by very poor diet with prolonged diœstrous intervals, but no change in cornified stages.

The foregoing studies indicate that both quantity and quality of proteins must be adequate to permit proper reproduction in rats, mice, and guinea pigs, and, probably, in other animals. Quantity of proteins was not determined in our deficient diet nor in the complete ones; but it was doubtless deficient both in quantity and quality in the former and adequate in the latter.

Bryan and Gaiser (1932) reported that both diet and increased amounts of anterior pituitary hormone are factors in increasing growth rate in adolescent male rats, and that both at the optimum together give maximum growth response. Diet affected the response to anterior pituitary hormone.

Agduhr (1931*a*, *b*) found that, with dogs, cats, and white mice, addition of medicinal cod liver oil prevented œstrus and ovulation and, in some cases, affected the testes. Taken in connection with the destructive effect of red light upon vitamin D in irradiated ergosterol, discussed elsewhere, this suggests the possibility that cod liver oil with vitamin D may be antagonistic to red light in action on sex glands and sexual cycles and that in some way it is tied to the calcium balance so that vitamin D raises the calcium in the system and interferes with the anterior hypophysis, the ovaries, and, less often, the testes.

Taken together, the preceding studies indicate a profound influence of diet upon reproductive rhythm and capacity through the presence or absence of vitamins A, B, D, E, through general inanition from nutritional deficiency, and through different proportions of proper proteins, fats, and mineral salts. Such deficiencies appear to induce conditions in testes similar to those shown by starlings and ferrets in winter, with the germ-cells limited to spermatogonia. These end results are induced in different ways by the different types of deficiency. Inadequate diets prevent or limit the action of anterior pituitary hormones much as they prevented or reduced the effect of red light in these experiments with starlings.

It is of interest here that Küstner (1932) found that anterior pituitary gonadotropic hormones were apparently destroyed by ultraviolet light, while their effectiveness was intensified by radiation with red light, so that the Aschheim-Zondek test for the presence of the gonadotropic hormones in urine could be read off in immature mice after 60–72 hours if the mice were kept in red light during the test, instead of the usual 96 hours under ordinary lighting conditions. Tests indicated that the hormone was present in both active and inactive forms in prolan, and that red light converted the inactive into the active form, increasing the active amount, and that ultra-violet rays reversed this action without destroying the hormone.

Whether the normal effect of added red light on starlings is to convert inactive gonadotropic hormone into its active form, thereby increasing its effect upon the gonads and indirectly on the accessory sex-organs, or to stimulate the hypophysis to increased secretion or liberation of the hormone into the blood stream to be effective, still remains an open question requiring further analysis. But that this effect is lessened by a restriction of the diet is beyond serious question.

Summary and Conclusions

1. The effects on testis activity of red light at about 1.7 foot candles for 6 hours per night with starlings on a complete and varied diet are

compared with those of red light at about 2.66 foot candles for the same time per night on similar birds fed a diet restricted to "middlings mash," poor in proteins, fats, vitamins, and mineral salts, and with controls on the complete diet without added red light.

2. The experiments were carried out in January and February for 22, 23, and 25-day periods.

3. On the complete diet, red light at 1.7 foot candles for 23 days led to large increase in testis size and in tubule cross-section, with metamorphosing sperms, in January; and, in February, with the same bulbs at slightly weakened intensity due to use, to a slightly less increase and to less advanced germ-cell stages, in 25 days.

4. On the restricted diet, the increase in testis size, tubule section, and spermatogenic activity was relatively slight, leading at most to multiplication of spermatogonia.

5. This is taken to indicate that restricted diet is a factor limiting the effectiveness of the light stimulus, or response to it, so far as sexual activity in male starlings during the long winter period of quiescence is concerned.

6. Cases from the literature are cited, in which lack of vitamins, proteins, fats, and mineral salts, either singly or in combination, has interfered with sexual activity or with the action of growth and gonadotropic hormones of the anterior pituitary, and their bearing on these experiments discussed.

7. Cases are cited and discussed in which long-waved light has increased the amount and effectiveness of anterior pituitary hormones and short-waved rays have decreased them.

8. It is concluded that increase of exposures to long-waved light can only be completely effective in inducing sex-gland activity, in starlings and at least some other animals, when the diet is rich in vitamins, proteins, and fats, as well as carbohydrates. The relative effects of deficiencies in each of these different requirements were not investigated.

LITERATURE CITED

ABERLE, S. B. D., 1933. Nutritional Inhibitors of Hormonal Action. *Anat. Rec.*, **55**: Supplement, p. 2.

AGDUHR, E., 1931*a*. Concerning the Influence of Diet on the Procreative Faculty of White Mice. *Proc. Sec. Intern. Congr. f. Sex. Res. London*, 1930, pp. 20–26.

AGDUHR, E., 1931*b*. Concerning the Influence of Diet on the Procreative Faculty of Dogs and Cats. *Proc. Sec. Intern. Congr. f. Sex. Res. London*, 1930, pp. 27–37.

AGNOLI, R., 1930. L'ormone ipofisario lipoideo e la vitamina della fertilità. *Boll. Soc. Ital. Biol. Sperim.*, **5**: 937. Cited from *Biol. Abstr.*, **6**: 1628.

BAKER, J. R., AND R. M. RANSON, 1932. Factors Affecting the Breeding of the Field Mouse (Misrotus agrestis). Part II. Temperature and food. *Proc. Roy. Soc.*, Ser. B, **112**: 39.

Bates, G. L., 1908. Observations regarding the Breeding-seasons of the Birds in Southern Kamerun. *Ibis*, **9**: 558. Cited from Bissonnette, 1932*b*.

Bissonnette, T. H., 1930*a*. Studies on the Sexual Cycle in Birds. I. Sexual maturity, its modification and possible control in the European starling (Sturnus vulgaris). *Am. Jour. Anat.*, **45**: 289.

Bissonnette, T. H., 1930*b*. Studies on the Sexual Cycle in Birds. III. The normal regressive changes in the testis of the European starling (Sturnus vulgaris) from May to November. *Am. Jour. Anat.*, **46**: 477.

Bissonnette, T. H., 1931*a*. Studies on the Sexual Cycle in Birds. IV. Experimental modification of the sexual cycle in males of the European starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work. *Jour. Exper. Zoöl.*, **58**: 281.

Bissonnette, T. H., 1931*b*. Studies on the Sexual Cycle in Birds. V. Effects of light of different intensities upon the testis activity of the European starling (Sturnus vulgaris). *Physiol. Zoöl.*, **4**: 542.

Bissonnette, T. H., 1932*a*. Studies on the Sexual Cycle in Birds. VI. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling (Sturnus vulgaris). *Physiol. Zoöl.*, **5**: 92.

Bissonnette, T. H., 1932*b*. Light and Diet as Factors in Sexual Periodicity. *Nature*, **129**: 613.

Bissonnette, T. H., 1932*c*. Modification of Mammalian Sexual Cycles. Reactions of Ferrets (Putorius vulgaris) of Both Sexes to Electric Light Added after Dark in November and December. *Proc. Roy. Soc.*, Ser. B, **110**: 322.

Bissonnette, T. H., 1932*d*. Light or Exercise as Factors in Sexual Periodicity in Birds. *Science*, **76**: 253.

Bissonnette, T. H., 1933. Light and Sexual Cycles in Starlings and Ferrets. *Quart. Rev. Biol.*, **8**: 201.

Bissonnette, T. H., and M. H. Chapnick, 1930. Studies on the Sexual Cycle in Birds. II. The normal progressive changes in the testes from November to May in the European starling (Sturnus vulgaris): an introduced, non-migratory bird. *Am. Jour. Anat.*, **45**: 307.

Bissonnette, T. H., and A. P. R. Wadlund, 1931. Spermatogenesis in Sturnus vulgaris: Refractory Period and Acceleration in Relation to Wave Length and Rate of Increase of Light Ration. *Jour. Morph.*, **52**: 403.

Bissonnette, T. H., and A. P. R. Wadlund, 1932. Duration of Testis Activity of Sturnus vulgaris in Relation to Type of Illumination. *Jour. Exper. Biol.*, **9**: 339.

Bissonnette, T. H., and A. P. R. Wadlund, 1933. Testis Activity in Sturnus vulgaris. Relation to Artificial Sunlight and to Electric Lights of Equal Heat and Luminous Intensities. *Bird Banding*, **4**: 8.

Bryan, A. H., and D. W. Gaiser, 1932. The Influence of Diet and the Anterior Pituitary Growth Hormone on the Growth Rate of Adolescent Rats. *Am. Jour. Physiol.*, **99**: 379.

Burr, G. O., and M. Sutermeister, 1932. Effect of Light on Rats Receiving a Complete Diet. *Proc. Soc. Exper. Biol. and Med.*, **29**: 1035.

Byerly, T. C., H. W. Titus, and N. R. Ellis, 1933. Production and Hatchability of Eggs as Affected by Different Kinds and Quantities of Proteins in the Diet of Laying Hens. *Jour. Agr. Res.*, **46**: 1.

Clayton, M. M., 1930. The Comparative Value of Different Food Proteins for Reproduction and Lactation in the Rat. II. Milk, egg, meats. *Jour. Nutr.*, **3**: 23.

Coward, K. H., 1929. The Influence of Vitamin A-Deficiency on the Oestrous Cycle of the Rat. I. *Jour. Physiol.*, **67**: 26.

Coward, K. H., B. G. E. Morgan, and F. J. Dyer, 1930. The Influence of Vitamin A-Deficiency on the Oestrous Cycle of the Rat. II. *Jour. Physiol.*, **69**: 349.

Evans, H. M., and K. S. Bishop, 1922*a*. On the Relations between Fertility and Nutrition. I. The ovulation rhythm in the rat on a standard nutritional régime. II. The ovulation rhythm in the rat on inadequate nutritional régimes. *Jour. Metabol. Res.*, **1**: 319–333, 335–356.

Evans, H. M., and K. S. Bishop, 1922*b*. On an Invariable and Characteristic Disturbance of Reproductive Function in Animals Reared on a Diet Poor in Fat-soluble Vitamin A. *Anat. Rec.*, **23**: 17.

Hill, M., and A. S. Parkes, 1930. The Effects of Anterior Pituitary Preparations on the Anoestrous Ferret. *Jour. Physiol.*, **69**: xviii and xix.

Hoffman, C., 1923. Reproduction of Animals on an Exclusive Diet of Bread. *Ind. Eng. Chem.*, **15**: 1225.

Kudrjaschov, B. A., 1930. Das Vitamin E und die Produktion des Testikularhormons. *Endokrinol.*, **7**: 91. Cited from *Endocrinology*, **15**: 552.

Küstner, H., 1932. Haben Lichtstrahlen einen Einfluss auf die Hormone und daren Wirkung im Tier und Pflanzenreich? *Zeitschr. f. Geburtsh. u. Gynakol.*, **103**: 305. Cited from *Jour. Organotherapy*, **17**: 169.

Lin, K. H., 1931. Nutritive Value of Vegetarian Diets from Economic Standpoint. *Nat. Med. Jour. China*, **17**: 200.

Lin, K. H. C. Tsai, and S. Wan, 1932. Estrual Rhythm of Vegetarian and Omnivorous Rats. *Chinese Jour. Physiol.*, **6**: 23.

Macy, I. G., J. Outhouse, M. L. Long, and A. Graham, 1927. Human Mlik Studies. I. Technique employed in vitamin studies. *Jour. Biol. Chem.*, **73**: 153.

Marrian, G. F., and A. S. Parkes, 1929. The Effect of Anterior Pituitary Preparations Administered during Dietary Anœstrus. *Proc. Roy. Soc.*, Ser. B, **105**: 248.

Mason, K. E., 1933. Differences in Testis Injury and Repair after Vitamin A-Deficiency, Vitamin E-Deficiency, and Inanition. *Am. Jour. Anat.*, **52**: 153.

Moreau, E. R., 1931. *Ibis*, Ser. 13, **1**: 553–570. Cited from Bissonnette, 1932*b*.

Ortiz, O., 1931. Funzione del testiculo e beri-beri sperimentale nel gallo. *Archivio di Sc. biol.*, **16**: 245.

Parkes, A. S., and J. C. Drummond, 1926. The Effects of Fat-soluble Vitamin A-Deficiency on Reproduction in the Rat. *Jour. Exper. Biol.*, **3**: 251.

Russell, W. C., 1932. The Addition of Raw Beef or Meat Scrap to a Wheat-milk Diet. *Jour. Nutr.*, **5**: 347.

Slonaker, J. R., 1931. The Effect of Different Percents of Protein in the Diet. IV. Reproduction. *Am. Jour. Physiol.*, **97**: 322.

Sorour, M. F., 1923. Versuche über Einfluss von Nahrung, Licht, und Bewegung auf Knockenentwickelung und endokrine Drüsen junger Ratten mit besonderer Berucksichtigung der Rachitis. *Beitr. z. path. Anat. u. Allgem. Path.*, **71**: 467. Cited from *Jour. Organother.*, **9**: abstr.

Sure, B., 1924*a*. Dietary Requirements for Reproduction. I. The nutritive value of milk proteins from the standpoint of reproduction. II. The existence of a specific vitamin for reproduction. *Jour. Biol. Chem.*, **58**: 681.

Sure, B., 1924*b*. Dietary Requirements for Reproduction. III. The existence of the reproductive dietary complex (vitamin E) in the ethereal extracts of yellow corn, wheat embryo, and hemp seed. *Jour. Biol. Chem.*, **62**: 371.

Sure, B., 1926*a*. Dietary Requirements for Reproduction. V. The rôle of various vegetable and fruit oils in fertility and lactation. *Jour. Biol. Chem.*, **69**: 29.

Sure, B., 1926*b*. Dietary Requirements for Reproduction. VI. Types of sterility produced on a skimmed milk powder reproduction-deficient diet. VII. The existence of a lactation-promoting factor in the unsaponifiable matter from wheat oil. *Jour. Biol. Chem.*, **69**: 41.

Sure, B., 1933. Dietary Requirements for Fertility and Lactation. XXV. Does the amount of fat in the diet influence vitamin B requirements for lactation? *Proc. Soc. Exper. Biol. Med.*, **30**: 622.

Swanson, P. P., and P. M. Nelson, 1933. Inability of the Albino Rat to Bear and Rear Young on Rations Supposedly Adequate in all Respects in which Canned Pork Muscle Serves as the Dietary Protein. *Am. Jour. Physiol.*, **105**: 92.

Vezár, F., 1931. Die Wirkung von E-Vitamin auf die Hypertrophie des Uterus (Inkretion und Avitaminose). *Pflügers Arch.*, **227**: 499.

Vezár, F., and E. V. Kokas, 1931. Die Wirkung des Mangels an E-Vitamin auf das Haarkleid der Ratten (Inkretion und Avitaminose). *Pflügers Arch.*, **227**: 511.

Wolfe, J. M., 1930. Effect of a Diet Low in Salts on Œstrous Cycle of Albino Mouse. *Proc. Soc. Exper. Biol. Med.*, **27**: 333.

Wu, H., and T. T. Chen, 1929. Growth and Reproduction of Rats on Vegetarian Diets. *Chinese Jour. Physiol.*, **3**: 157.

CONDITIONS AFFECTING THE RESPONSE OF THE AVICULARIA OF BUGULA

ALEXANDER FORBES

(*From the U. S. Bureau of Fisheries and the Marine Biological Laboratory, Woods Hole, Massachusetts*)

Introduction

The avicularia, or "bird's heads," on the *Bugula* which abound in the waters around Woods Hole present an interesting example of primitive muscular mechanism—interesting both from the point of view of physiology and from that of their utility to the colonies in which they are found. Two species of *Bugula* (*B. flabellata* and *turrita*) are common in this vicinity. Each zoöid consists of a shell into which the polypid with its ring of ciliated tentacles can on appropriate stimuli be retracted. Normally each zoöid has also an avicularium growing on the outside of its shell as an appendage apparently quite distinct from the rest of the organism. Indeed active avicularia are sometimes found on dead zoöids in which the rest of the organism is already decomposed. The avicularium has an extraordinary resemblance to the head of a hawk or eagle, the hooked beak and the articulation of the lower jaw or mandible being almost identical in shape with those of the bird (Figs. 1 and 2). Usually the beak is held wide open, the angle between the jaws being about 150°. To quote Harmer (Cambridge Natural History, Vol II, p. 484), ". . . A great part of the head is filled with a strong muscle, whose fibers exhibit a distinct transverse striation, and converge into a median tendon. . . . The muscle serves to close the jaws. . . . The lower jaw is opened by means of a pair of muscles. . . ."

"Within the jaws, in the region which we may term the palate, is a rounded knob, which bears a tuft of delicate sensory hairs, which doubtless enable the avicularium to recognize the presence of any foreign body. The closure of the mouth may, indeed, be instantaneously induced by touching it with the point of a needle."

When a branch from a colony is placed in sea water under a microscope the avicularia are seen to sway back and forth on their stalks in the sagittal plane, usually remaining from two to ten seconds in the backward position and five to twenty seconds in the forward position; occasionally the entire cycle is as brief as five seconds or less. At intervals of a minute or two the beak closes with a quick snap, and after remaining closed for from one to five seconds or so, opens again, whereupon the avicularium continues swaying as before. Usually the closure occurs during the forward swing of the swaying motion. As far as was observed, the swaying always ceases while the beak is closed.

As to the biological significance of this structure, Hertwig (p. 323) states that it has been seen to seize and hold small animals, and suggests that its function is to feed the colony. In Parker and Haswell (p. 352) a defensive function is suggested. Harmer (*loc. cit.* pp. 485–487) says, "In the course of some observations . . . on *Bugula calathus* at

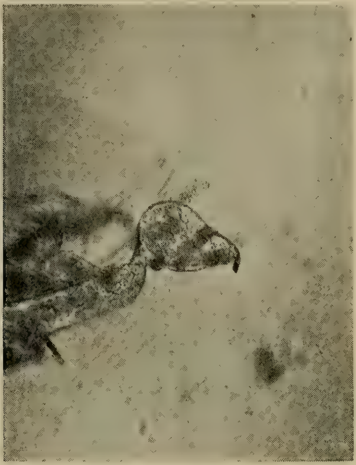

FIG. 1. Microphotograph of avicularium when beak had been closed and swaying stopped by injection of fresh water. The branch of the colony was broken close to the attachment of the avicularium; this afforded an exceptionally clear background.

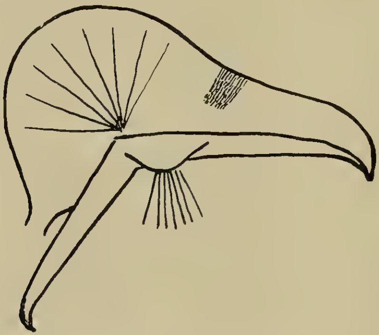

FIG. 2. Diagram of avicularium with beak open, showing tuft of hairs on rounded knob. The position of the muscle in the crown of the head is shown by radiating lines.

Naples, a fine hair offered to a small colony was seized with such force by the avicularia that the entire colony was lifted out of the water by the hair. The same colony had captured (1) a small *Nereis*, which it held with several of its avicularia; (2) an Anisopod Crustacean, $2\frac{1}{2}$ mm. long; and (3) a small Amphipod, which was held by one of its antennæ. . . . Curiously enough, however, an avicularium did not necessarily close even when part of a captured animal was actually in its mouth. The avicularia made no attempt to place themselves in an advantageous position for catching fresh parts of the *Nereis*, which they might easily have done. The avicularia which had captured prey remained motionless. The others moved backwards and forwards . . . ten times in 3/4 to 1 minute, snapping their jaws perhaps once in that time. The two Crustacea were still retained by the avicularia two days later. On the next day they had both disappeared; but the colony had again caught the *Nereis*, which had previously effected its escape with the loss of nearly all its tentacular cirri . . . it cannot be doubted that the avicularia can prevent inquisitive worms from straying at will over the surface of the colony."

In 1905, in the laboratory of the U. S. Fish Commission at Woods Hole, an attempt was begun in collaboration with Dr. E. B. Krumbhaar, to ascertain the nature of the stimuli and environmental conditions which give rise to the closure of the beak. The speed of closure is characteristic of the contraction of striated muscle. The question arises as to how the muscle is caused to contract. Bearing in mind the theory that the avicularia aid in feeding the colony, we selected a nutrient material for our first test. A branch from a colony was placed in sea water in a shallow glass dish under a microscope and the avicularia were watched for some time. One selected for observation was seen to snap shut at intervals of approximately 100 seconds. A mussel was then broken open and some of the fluid from its shell drawn into a fine glass pipette. This material was then squirted into the water surrounding the avicularium under observation. Immediately the interval between successive closures fell to about 8 seconds, but soon increased again to approximately its previous value.

As a control, sea water was similarly squirted at the structures under observation, in order to ascertain whether the effect had been due to the current of fluid. No increase in frequency of closure appeared to follow this procedure.

In another test, a solution of cane sugar was injected into the field. No response to this procedure was observed.

Further experiments have been conducted at the Marine Biological Laboratory at intervals since the preliminary observation, in the quest for more light on the problem. The material has been *Bugula turrita*, usually scraped from the piles of the wharf, but in some experiments small colonies grown in the laboratory on glass slides by Dr. Young were used. The latter had the advantage of freedom from foreign material with which the colonies are usually encumbered.

METHOD

The method which proved most satisfactory was to break a small branch from the colony, large enough to include a few dozen zoöids, and place it on the floor of a Stender dish with a small glass weight resting on the stem or base of the branch to steady it and keep the zoöids under observation fairly still in the microscope field while the test substance was being introduced. Under a low power microscope one or more of the avicularia were observed and their activity when undisturbed was carefully noted. Attention was chiefly focussed on the frequency of closure, but in some of the experiments the character of the swaying was also noted.

After the effect of the test substance had been observed it was freely washed out with sea water and the material was examined until it was evident that typically normal conditions were re-established before another test was made.

The time of closure and opening was usually recorded by rapid reference to the second hand of a watch. In a few experiments it was recorded on a smoked drum with a signal magnet operated by hand. This procedure enabled the observor to watch the material without interruption.

RESULTS

As already stated, in the first experiment mussel juice strikingly increased the frequency of closure. In many subsequent experiments the same procedure produced the same result with perfect regularity. A typical example follows. A field was found in which 10 avicularia could be seen at once. Of these, 2 held their beaks closed; the remaining 8 were swaying with their beaks open. Occasionally one was seen to snap shut. Later when 7 were in the field, 3 or 4 of them were observed to snap during a 50-second interval while they were continuously watched. After this, mussel juice, drawn directly from the shell, was injected. Apparently all avicularia in sight snapped within 1 or 2 seconds of the time the injection entered the field. A single avicularium was then watched and the times of successive closures recorded as follows: 8, 8, 10, 15, 20 seconds. Two or three minutes later the frequency of closure was still above normal. Twelve minutes after the injection the frequency had returned approximately to normal.

Another effect of these injections was a marked increase in the swaying of the avicularia. Similar results were frequently obtained with the juice of the clam and quahog. In two experiments fresh cow's milk was injected. In one of these an apparently positive result of increased frequency of snapping was observed; in the other, no effect. Again dilute solutions of sugar in sea water appeared to be wholly without effect.

In one experiment a solution of 20 per cent ethyl alcohol and sea water was injected. The injection was immediately followed by vigorous withdrawal of the tentacles of the polypids into their shells. No closure of the avicularia was observed, and about a minute after the injection all motion ceased. The avicularia had stopped swaying, and all the beaks remained wide open.

The injection of a solution of sodium chloride of much higher concentration than sea water was followed by closure of the beaks and cessation of swaying. Instead of opening as usual, the beaks remained closed. After 5 minutes the salt solution was washed away with fresh sea water, and 2 minutes later the avicularia were again swaying in normal fashion and occasionally snapping shut as usual. In a number of subsequent experiments this effect was tested by merely concentrating the sea water by boiling down to about 40 per cent of its original volume. Considerable volumes of this concentrated sea water were injected into the field of observation. Since the injections were diluted by the sea water in which the preparation was immersed, it was impossible to make these observations quantitative. It was evident, however, that with strong concentrations the avicularia regularly closed their beaks, stopped swaying and remained motionless with their beaks closed for an indefinite time. With weaker concentrations a fairly rapid series of closures would be induced, similar to those which followed injections of mussel juice. Sometimes after two or three closures the beak would remain closed as in the case of stronger injections. In other cases, after remaining closed for half a minute or so, the beak would gradually open and resume its normal activity. This effect is presumably explained as the result of return to normal concentration of sea water through diffusion.

In three experiments the effect of dilution was tried by injecting fresh water into the field. The effect was somewhat similar to that of concentration, in that a small quantity of fresh water seemed to cause frequent closures of the beak, whereas larger quantities caused the beaks to close and remain closed and motionless (Fig. 1). As in the case of concentrated salt solution, even after the beaks had remained closed and motionless for several minutes, they could be made to open and resume their normal swaying by returning them to sea water.

Chemical Stimuli

It was suggested by Professor G. H. Parker that the effects of various ions should be investigated. The following substances were tried:—acid (HCl), alkali (NaOH), $MgSO_4$, $CaCl_2$ and KCl. None of these produced such striking effects on the avicularia as did the juice of the mussel and clam. In some experiments the activities of the tentacles of the polypids, as well as those of the avicularia, were noted. The results are shown in Table I.

TABLE I

Reagent	Concentration of injected fluid	Effect on avicularia	Effect on polypid tentacles
NaOH	0.002 N	Slightly increased swaying, no closure.	Not noted.
	0.1 N	Delayed permanent closure.	Activity, then retraction.
HCl	0.001 N	No effect.	Increased activity.
	0.002 N 0.01 N	Rapid closure, then opening with cessation of swaying.	Not noted.
	0.02 N	Closure, followed by slow incomplete opening.	Not noted.
	0.1 N	Rapid permanent closure.	Retraction.
$MgSO_4$		Slight, doubtful increase in frequency of closure.	Not noted.
$CaCl_2$	Concentrated.	Doubtful increase in closures.	Not noted.
	Dilute.	Slight, doubtful increase in closures.	Not noted.
KCl	1 gram in 20 cc. sea water.	No closure, cessation of swaying.	Not noted.
	Diluted till not refractile in sea water.	Rapid closure, then opening with cessation of swaying.	Retraction.

It should be noted that the concentration given is that of the injected fluid, which necessarily became diluted to an unknown extent on injection into the field. In the case of $CaCl_2$, its solution in sea water resulted in the formation of crystals. To avoid mechanical effects, these were allowed to settle and the clear fluid above was used. This appeared highly refractile when dropped into sea water, and in some experiments, in order to minimize osmotic effects, it was diluted with fresh water till the refractile effect disappeared.

The most striking result in this series was the effect common to alcohol, KCl, and weak HCl. Whereas normally the avicularia only cease swaying when the beaks are closed, these three reagents caused cessation of swaying with the beaks open.

In one experiment some of the mussel juice was tested with cresol red and found to have a pH of about 7.4. The pH of sea water, measured with thymol blue, was found to be about 8.2 to 8.4. From this it might be questioned whether part of the reaction of the avicularia to mussel juice could be due to its acidity. The differences in the reactions to the two agents, described above, renders this highly improbable.

Several experiments were tried with solutions of egg albumen. The albumen was stirred up in about twice its volume of sea water. After stirring it did not appear to go completely into solution, but fluid drawn from the upper layer, *i.e.* above the albumen lying visibly in the bottom of the dish, when dropped into sea water showed distinct refractile effect, and greater density than sea water, by sinking to the bottom. Injection of this solution caused a marked positive response. Several avicularia started closing fairly regularly at intervals of from 3 to 15 seconds and continued to do so for some time. This procedure was repeated several times in different experiments and always produced similar results. In one instance the increased frequency of closure was observed for as long as 4 minutes after the injection. This persistence of the increased frequency of closure seemed to place the response in the class with the response to injections of mussel juice, rather than with the response to concentrated sea water, whose effect, when marked, terminated with permanent closure and cessation of activity. In one experiment the solution of egg albumen and sea water was diluted with fresh water till it appeared less dense and slightly less refractile than sea water. This diluted solution appeared to cause a slight doubtful response in a few avicularia somewhat similar to that of the potassium chloride. Some of the albumen solution was then carefully mixed with fresh water till it seemed to have about the same density as sea water. An injection of this solution was followed by occasional closures of one or two avicularia, but again it was doubtful if this was a true positive response. Certainly it was not nearly so marked as that produced by a dense solution of albumen.

To eliminate the possible effect of solids, some of the mussel juice was filtered. The filtrate caused a visible refractile effect when dropped in sea water. This filtrate, when injected into the field caused in the avicularia a positive response almost as pronounced as that which occurred when mussel juice with all its solids was injected. *Limulus* blood injected into the field caused no apparent effect. In another test, filtered *Limulus* blood was injected. Within 40 seconds 3 avicularia were seen to snap, which suggested a positive response, but must be considered doubtful. Apparently the most pronounced positive response is caused by solutions having a marked density, such as the juice of the mussel and the clam or fairly concentrated solutions of albumen.

That this effect may be due in part to a high osmotic pressure of the solution is suggested by the fact that closure is induced by concentrated salt solution. On the other hand, since the response to strong salt solution usually differs from that evoked by albumen, in that the result is usually a permanent closure or at least a closure more prolonged than normal, rather than an acceleration of the frequency of normal closures, and since albumen solution is characterized more by density than by a high osmotic pressure, it may be inferred that the mass of the molecules may be a significant factor rather than the osmotic pressure.

Thermal Stimuli

The effect of heat was tested by injecting sea water warmed to between 28° and 36° C. This caused acceleration of swaying, and possibly a slight increase in closure.

Electrical Stimuli

Electrical stimuli were tried in several experiments. Electrodes were applied to opposite sides of the field of observation. In some experiments galvanic currents from a 3-volt battery were employed; in others faradic stimuli from the secondary coil of an inductorium, rapidly interrupted (tetanizing) currents being delivered by means of a vibrating armature. Neither galvanic nor faradic currents of any strength employed appeared capable of stimulating the closing muscle of the avicularium. The beginning of the constant current or of the series of induction shocks was always promptly followed by retraction of all the polypids into their shells, but the avicularia seemed to continue swaying without giving any visible response. On one occasion when the current was strong enough to liberate bubbles of gas in the sea water one avicularium was seen to close when one of the electrodes was brought very close to it. On another occasion faradic stimulation, much stronger than was required to induce retraction of the polypids, appeared to cause a slight delayed response in the avicularia. Since in no instance did the closure of the beak follow immediately on application of the electrical stimulus, but if at all, only after a strong current had been flowing for several seconds, it may be inferred that such stimulation as occurred was due to the electro-chemical effects of the current passing through the sea water and not to a direct electrical excitation of the muscle. It is remarkable that this striated muscle, which is so readily responsive to certain types of stimuli, should be so refractory to electrical stimuli whose effect on the muscles of the adjacent polypids was so marked.

Mechanical Stimuli

It was only after most of the experiments thus far described had been performed that I became aware of the existence of the tuft of hairs within the angle of the jaws, described by Harmer. These structures are small and can only be seen upon careful examination with fairly high power. The opinion expressed by Harmer, that these hairs are sensory receptors for tactile stimuli, suggested a new line of inquiry. The most effective substances thus far tried had been the juice of the mussel and the clam, which contained a considerable amount of solid material whose contact with the hairs might have caused the response.

Two procedures were adopted to test the question whether the hairs might serve as receptors for tactile stimuli or indeed might prove to be the only effective means of evoking a response in muscle. One was the comparison of the effects of the entire mussel juice, including its solids, with that of the filtrate, from which the solids had been removed. The other was to introduce insoluble solids which could fairly be assumed to be chemically inert. The result of the first procedure has already been described. Filtered mussel juice proved effective in increasing the frequency of closure of the avicularia, although probably not quite so effective as the whole mussel juice including the solids. From this and from the effect of albumen solution it may be inferred that mechanical contact of solids with the hairs is not the only possible source of stimulation.

In several experiments the direct effect of inert solids was tried. The substances employed were suspensions of clay which had been washed repeatedly in sea water to remove any soluble substances which might confuse the effect, and MgO, the crystals of which are apparently quite insoluble in sea water. A considerable number of injections of clay were made, and a majority of these were followed quite promptly by closure of one or more avicularia under observation. A few tests were negative, but a majority seemed to show a fairly definite positive response. A certain percentage of negative results in such a test is to be expected, since it is a matter of chance whether any particles of clay come in contact with the hairs in a particular avicularium.

Several tests were made with suspensions of MgO crystals. In some ways this material was more satisfactory than clay, since the crystals formed a more even suspension with less tendency to obscure the field. The fairly rapid rate with which they sank facilitated observations in the following manner. A drop of the suspension was let fall from a pipette directly over the field of observation. The crystals would then sink in a few seconds to the bottom, and the moment at which they reached the focal plane of the microscope, including the avicularia under observation, could be observed and the effect noted. Several times this procedure produced a fairly striking positive result, of which the following is typical. When a cloud of crystals reached the focal plane, 4 out of 5 or 6 avicularia in the field snapped almost simultaneously and 3 of them repeated snapping at intervals of 10 seconds or less thereafter. When the MgO had been freely washed away, the avicularia returned to their usual slow frequency of closure. In some experiments no response to the injection of MgO crystals was seen, although in at least one instance the crystals appeared to come in contact with the hairs as the avicularia swayed forward. The failure in these cases may well be explained on the supposition that the mass of the crystals which collided with the hairs in these instances was too small to provide an adequate stimulus. In one experiment the crystals which formed when anhydrous $CaCl_2$ was dissolved in sea water appeared to produce a similar positive effect on two of the avicularia in the field.

In spite of the difficulty of insuring positive mechanical stimulation by the injection of solids, enough instances of response followed these injections to warrant the conclusion that solid masses in some way tend to cause closure of the beaks. The best evidence of this sort came through a fortunate accident. In one experiment, while an avicularium was well placed in the field for careful observation, a small planarian was seen to crawl along the branch of the colony. As the worm approached the avicularium its anterior end was seen to come squarely in contact with the tuft of hairs in the angle of the wide open jaws. Instantly the jaws snapped shut upon the head of the worm. The immediate response to this contact seemed to leave little doubt that the hairs had indeed acted as receptors to the tactile stimulus, thus confirming the assumption expressed by Harmer, as did occasional closures on contact with other foreign bodies. Clearly contraction of the closing muscle can be evoked by adequate mechanical disturbance of the tuft of hairs within the jaws.

SUMMARY

1. Attempts have been made to throw light on the function of the avicularia of *Bugula* by noting the effect of various physical and chemical agents on their activity. Both concentration and dilution of sea water caused prolonged closure of the beaks, with cessation of swaying. Slight concentration and dilution caused rhythmic closing and opening of the beaks, the periods of closure being relatively longer than normal. Strong alkali (NaOH) and acid (HCl) caused lasting closure, although in the case of alkali the reaction was less prompt than with acid or concentrated sea water. Weak alkali accelerated the swaying, but did not cause closure. Weak acid caused rapid closure followed by opening and cessation of swaying. Inorganic salts did not cause any pronounced reaction, although KCl caused a response similar to weak HCl—a single closure followed by cessation of closure and of swaying of the avicularia, the beaks remaining open.

2. The fluid drawn from the shell of the clam or the mussel regularly caused a great increase in the activity of the avicularia, and in the frequency of closure. Albumen solution caused nearly as marked a reaction, but its effect largely disappeared when the solution was diluted to the same density as the sea water in which the *Bugula* lay. Sugar solution produced no effect. The effect of mussel juice was not wholly due to solids in suspension, since it was nearly as marked in the case of the filtrate from which the solid particles had been removed.

3. Mechanical stimuli due to injections of inert solids in suspension in sea water are effective in causing closure. These stimuli probably act on the tuft of hairs in the angle of the jaws. This was strikingly shown by the prompt closure when the head of a worm came in contact with the hairs. Harmer reports a similar response to touching them with a needle.

4. No response could be produced by electrical stimulation, even when the current caused all the polypids to be promptly retracted. A slight delayed response occasionally following a very strong stimulus was probably due to electrochemical effects.

BIBLIOGRAPHY

GRAVE, B. H., 1930. The Natural History of Bugula flabellata at Woods Hole, Massachusetts, including the Behavior and Attachment of the Larva. *Jour. Morph. and Physiol.*, **49**: 355.

HARMER, S. F., 1901. Cambridge Natural History, Vol. II. Polyzoa, pp. 463–533.

HERTWIG, R., 1909. Manual of Zoölogy. Henry Holt Co., New York.

PARKER, T. J., AND W. G. HASWELL, 1928. A Text-Book of Zoölogy. Macmillan & Co., Ltd., London.

MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

III. The Distribution of Bacteria in the Ocean Waters and Muds About Cape Cod[1]

HERBERT W. REUSZER[2]

Introduction

A considerable number of investigations concerning the number of bacteria and their distribution in sea water have been made. Among the earliest of these was that of Russell (1892, *a* and *b*, 1893), who conducted systematic studies of the distribution of bacteria in the Gulf of Naples. He found bacteria in small numbers to be uniformly distributed in the water at distances of 3 to 15 kilometers from shore and at depths ranging from 50 to 1,500 meters. It was found by others (Cassedebat, 1894; de Giaxa, 1899) that while the water of harbors and bays might contain large numbers of bacteria, this number, as determined on the ordinary solid bacteriological media, became very small at comparatively short distances from shore. Bassenge and Fischer (1894, *a* and *b*), who investigated the water over a wide area of the Atlantic Ocean, found bacteria in larger numbers than have any other investigators. The largest numbers, however, were found near land or in the presence of large quantities of floating sea weed. Lloyd (1930), in her investigations in the Clyde Sea area, followed the distribution of bacteria at five stations during the course of a year. In the rather shallow waters of this region, the surface water was found to be richest in bacteria, the number decreasing with depth but usually increasing slightly again at the bottom. The numbers in the surface water fluctuated considerably but no definite seasonal trend could be detected; below the surface the numbers were remarkably constant throughout the year. Numerous, usually somewhat fragmentary investigations, ranging from polar to tropical waters, have almost invariably shown small numbers of bacteria to be present in the sea water. Among these may be cited those of Frankland and Burgess (1897), Levin (1899), Otto and Neumann (1904), Gazert (1912), Berkeley (1919) and Lipman (1929). The results of recent investigations such as those of Cholodny (1929) and Rasumow (1932), in the direct microscopic examination of sea water, indicate that bacteria are much more abundant than the cultural methods used by previous investigators have shown.

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[2] The author wishes to acknowledge the assistance of Mr. Charles E. Renn in certain of these experiments.

Studies concerning the bacteria of marine bottom deposits have been few. Russell (1892 *a*) studied the distribution of bacteria in the mud of the Bay of Naples simultaneously with his studies of bacteria in the water. This included mud at depths ranging from 50 to 1,100 meters below the surface of the water and extending to 15 kilometers from land. He found that down to depths of 200 meters, the number of bacteria decreased with distance from land, the range being from 70,000 to 285,000 bacteria per cubic centimeter of mud. Below 200 meters the number remained more constant. He had no satisfactory explanation to offer for this distribution. In work of the following year (1892 *b*) on the mud of Buzzards Bay on the Massachusetts coast, he found smaller numbers of bacteria than at Naples, a fact which he correlated with the lower water temperature in Buzzards Bay. The number of bacteria in sand bottom was much lower than in mud. Russell considered the mud to have a bacterial flora indigenous to itself, since two or three species of bacteria normally found only in the mud made up 30 to 50 per cent of all the bacteria present. Fischer (1894 *a*) and Gazert (1912) appear to be the only investigators who have studied the bacteria in deep sea deposits. Neither found bacteria to be very abundant in deposits of this nature. From many of their samples no bacteria developed. Their results are in direct contrast with those obtained from marine mud near shore. In the chalky mud of the flats about Andros Island in the Bahamas, Drew (1913) found bacteria to be present up to the number of 160,000,000 per cubic centimeter. Lipman (1929) and Bavendamm (1932) likewise found very large numbers of bacteria in these deposits. Lloyd (1931), in her systematic investigations in the Clyde Sea area, found bacteria up to 300,000 per gram of dry mud in the surface layer of mud. The number of bacteria decreased with depth and in the lower depths of the mud remained fairly constant. In the upper layers of mud there was in certain cases a bacterial zonation which was probably correlated with the stratification of the mud into organic and inorganic layers as noted by Schokalsky (1928) and Moore (1931).

The literature of marine bacteriology has recently been reviewed extensively by Benecke (1933), but the brief review above is sufficient to indicate that the work in general has been fragmentary in nature. There have been few studies of the distribution of bacteria in the sea in which an attempt was made to establish definitely the significant factors with which the observed changes in bacterial numbers might be associated. The supplementary data obtained by various investigators is in most cases so scanty as to make it impossible to correlate the results of one investigator with those of another.

The studies reported below represent preliminary results in a more extensive study of the occurrence of bacteria in the sea and the factors influencing their distribution. The areas investigated were the continental slope south and east of Cape Cod, the Gulf of Maine, and certain of the bays adjacent to Cape Cod. The station locations, with the exception of those in Buzzards Bay and the Gulf of Maine, are given in Fig. 1. The location of the Gulf of Maine stations has been previously given (Rakestraw, 1933).

EXPERIMENTAL

Methods

The water-sampling bottle used in this work was similar to the evacuated glass tube used by Russell (1892 *a*), the original idea of which is ascribed by him to Massea. For breaking the tip of the inlet tube at the proper depth, a modified form of the sampler described by Wilson (1920) was used. Mud samples were obtained by means of a modified form of the sampler described by Moore and Neill (1930). The glass tube in which the sample was secured was sterilized before use and the sample for analysis taken from the center of the core. The tube was closed at both ends with a sterilized rubber stopper immediately upon reaching the surface. No sampler satisfactory from the bacteriological standpoint is available for sampling sandy deposits. The few analyses of the bacterial content of such deposits reported in this paper were obtained from samples taken from the center of the material brought up in a sampler of the clam shell or snapper type.

In the present investigation, as in most previous ones, the plate method of determining the number of bacteria was used. It should be pointed out that since not all bacteria are capable of developing on the agar plate, the results so secured must be interpreted with considerable caution, particularly in regard to the total number of bacteria found. The observations of Pütter (1924), based on the oxygen consumption of the bacteria in sea water, and the work of Cholodny (1929) and Rasumow (1932) with the direct microscopic count, all indicate that the number of bacteria actually present in sea water is much greater than the cultural methods of determining their abundance have indicated. In preliminary experiments with various media, one having the following composition gave the highest counts of bacteria and was used throughout the work:

Peptone	1 gram
Glucose	1 gram
K_2HPO_4	0.05 gram
Agar	15 grams
Sea water	1000 cc.

It is interesting to note that with the exception of the addition of glucose, this medium is similar to the one employed by Drew (1913) and which Lipman (1929) found to give higher counts than any of the other media with which it was compared. Sea water was plated both undiluted and diluted ten times, three plates of each dilution being used. The mud was plated in a similar manner, except that the moist mud was diluted 10, 100, and 1000 times. Moisture determinations were made on the mud samples and the results are reported as number of bacteria per gram of dry mud. All samples, immediately upon being taken, were placed in a refrigerator at 4–5° C. and kept at this temperature until plated. In most cases the plating took place in less than twenty-four hours after taking the sample. The plates were incubated at room temperature and counted at the end of 7 and 10 days.

Abundance of Bacteria in Sea Water

In order to determine the variability which might normally be expected in the number of bacteria in sea water, 10 samples were taken at a single station, on the 20-fathom line south of Martha's Vineyard. All the samples were taken at a depth of 10 meters, and were plated out immediately when brought to the laboratory. The number of bacteria found in each of the 10 samples was as follows: 6, 2, 3, 4, 8, 83, 10, 24, 11, 27, 18. The average number of bacteria for all the 10 samples was 19 per cubic centimeter, with a variation between the individual samples of 2 to 83 cells per cubic centimeter. It would seem, therefore, that considerable variation may be expected in the number of bacteria in samples of sea water taken from the same spot. Russell (1893) observed that an occasional sample of sea water contained much larger numbers of bacteria than was to be expected from the bacterial content of the particular locality. A single species of bacteria always predominated in such samples. While the number of colonies appearing on plates of the same series was in general quite uniform, a similar observation was made during the course of the present work. The increased number of organisms sometimes was very large and occasionally appeared on only one plate of a series. Russell explained this phenomenon by assuming that some of the bacteria in sea water exist in the form of zoögleal masses. It suggests also, however, that part of the bacteria may be associated with particulate matter in the sea and that the large numbers of bacteria in certain samples may be due to the presence of the decomposing remains of some small marine organism such as a copepod or a chain of diatoms.

The data in Table I give an indication of the number of bacteria occurring in water receiving land and sewage drainage and illustrate the effect of local currents. Samples 1 and 2 were taken in the outer harbor of New Bedford and were very much higher in bacteria than were the three samples taken in Buzzards Bay outside the harbor. The location of these stations is not indicated on Fig. 1, but none of them were more than one or two miles from land. At the time the samples were taken the tide was rising and the direction of water movement was from Station 5 into the harbor towards Stations 1 and 2 and up the bay from Station 5 towards Station 3. The fact that the water, taken so near a source of pollution as was Sample 1, did not contain more bacteria is probably due to the fact that with an incoming tide at this time the tidal currents tended to carry the polluted water up into the harbor and left in its place the Buzzards Bay water from Station 5 with relatively low bacterial content. This is indicated by the fact that the water at Station 1, 100 yards on the bay side of the sewer outlet, contained 3,200 bacteria per cubic centimeter, while that at Station 2, approximately one mile away but in the direction of the water movement, contained 4,200 per cubic centimeter. The water in the bay itself contained much smaller numbers of bacteria. At Stations 4 and 5 there were about 100 bacteria per cubic centimeter, while

TABLE I

Abundance of bacteria in water and mud of New Bedford Harbor and Buzzards Bay, July 1, 1932.

Station	Location	Water depth	Number of bacteria per cc. of water or per gram of dry mud				Carbon content of surface mud	Carbon content of mud at 10 cm. depth
			Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm. depth		
		meters					*per cent dry mud*	*per cent dry mud*
1	100 yds. from sewer outlet	9	1790	3230	158,500	20,100	3.37	1.21
2	Across channel from sewer outlet	8	—	4250	41,200	—	0.58	
3	Above harbor entrance	15	—	197	79,900	—	2.30	
4	Directly opposite harbor entrance	10	—	94	48,000	44,400	1.96	
5	Below harbor entrance	18	22	111	34,000	—	1.76	

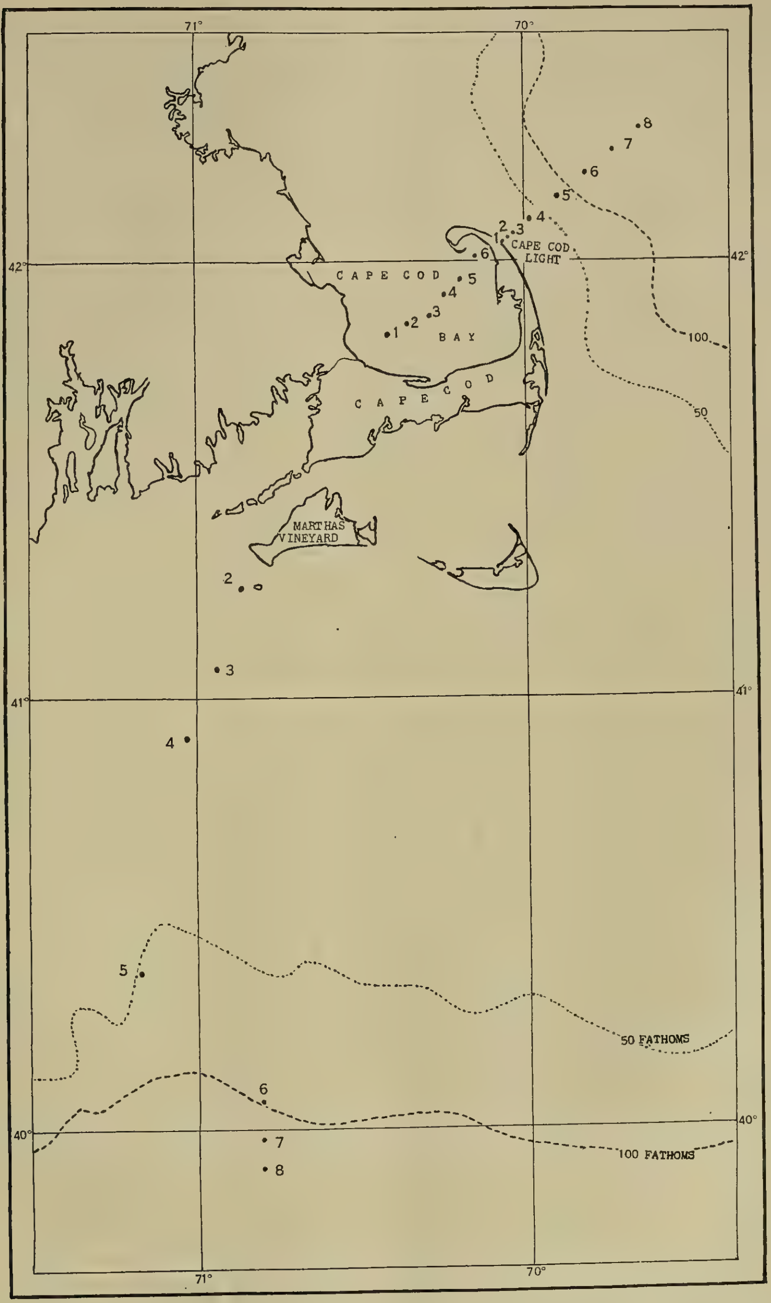
71°
70°
42°
41°
40°
CAPE COD BAY
CAPE COD
CAPE COD LIGHT
MARTHAS VINEYARD
1
2
3
4
5
6
7
8
100
50
50 FATHOMS
100 FATHOMS

FIG. 1. Chart showing location of the stations sampled for determination of the number of bacteria in sea water and bottom deposits.

at Station 3 farther up the bay the number reached 200 per cubic centimeter. The surface water evidently contained fewer bacteria than did the bottom water. An incomplete series of surface samples was secured because of loss of vacuum by the bottles prepared for taking them.

To secure information as to the number of bacteria in water and bottom deposits of the open ocean away from the influence of land a line of stations was sampled from the southern shore of Martha's Vineyard Island to the continental slope about 80 miles to the south. The results of these determinations are given in Table II. The number of bacteria in the surface water was found to vary irregularly and did not seem to be correlated with distance from land or depth of water. While the sample taken at 8:30 in the morning contained the highest number of bacteria, the sample with the second highest number of bacteria was taken at 6:00 P.M. There appeared to be no definite correlation between bacterial numbers and the length of time during which the surface water had been exposed to sunshine, as was suggested by Fischer (1894 *a*). The bottom water at the two stations at which samples were obtained was much higher in bacteria than the corresponding surface water. These samples were taken very near the bottom in water of no great depth, where agitation of the surface of the bottom by water movements may have carried bacteria into the lower layers of water.

TABLE II

Abundance of bacteria in sea water and in sea bottom deposits south of Martha's Vineyard, July 19–20, 1931.

Station No.	Depth of water	Distance from land	Hour of day when sample was taken	Number of bacteria per cc. of water or per gram of dry mud				Loss on ignition
				Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm.	
	meters	*miles*						*per cent dry mud*
2	18	1.5	7:00 P.M.	65	159	—	—	—
3	38	12	5:00 P.M.	183	1900	238 (sand)	—	0.69
4	55	23	3:30 P.M.	185	—	1050 (sand)	—	0.94
5	94	55	8:30 A.M.	735	—	7700	2700	7.93
6	163	72	7:00 P.M.	27	—	1000 (sandy)	—	3.17
7	345	77	6:00 P.M.	480	—	1400	460	6.14
8	570	81	3:00 P.M.	190	—	1280	900	7.24

A series of observations of the bacterial content of the water and mud off the eastern coast of Cape Cod north of the Nantucket Shoals and Georges Bank was made during the summer of 1932. This line of stations extends NE by E$\frac{1}{2}$E from Cape Cod Light, the sampling stations being 100 yards and 1, 2, 5, 10, 15, 20, and 25 miles from shore. As will be seen from the water depths, the ocean bottom along this line slopes gently downward to about 15 miles off shore, where it levels off at a fairly constant depth of about 245 meters. The number of bacteria found in the water of these stations at three different periods during the summer is recorded in Table III. With the exception of the samples taken near the beach, extremely few bacteria were present in the water at any time. A considerable number of the samples did not contain any bacteria capable of developing on the agar plate. The highest number found was 78 per cc. at Station 4 on August 24. The number of bacteria tended to be slightly higher at all stations at this date. The number of bacteria in water one mile from shore was no greater than in water taken farther from shore. On an exposed shore such as this with the sea receiving almost no land drainage, it is doubtful if the number of bacteria in the water is directly affected by the presence of land. The larger number of bacteria in water from the beach is no doubt due to bacteria carried up from the bottom by wave action.

Table III

Abundance of bacteria in sea water off Cape Cod Light, July 13–September 20, 1932.

Station No.	Distance from shore	Depth of water	Number of bacteria per cc. water					
			July 13		August 24		September 20	
			Surface water	Bottom water	Surface water	Bottom water	Surface water	Bottom water
	miles	*meters*						
1	100 yds.	5	38	2	394	793	—	—
2	1	25	0	1	—	—	—	—
3	2	36	0	0	—	—	—	—
4	5	83	1	0	78	22	—	—
5	10	159	3	0	1	1	—	—
6	15	243	0	1	12	3	0	—
7	20	246	4	0	5	—	0	1
8	25	245	—	—	5	2	0	0

The bacteria in the water off Cape Cod Light may be compared with the number found in Cape Cod Bay (Table IV) and also with those for the Gulf of Maine and Georges Bank. The latter results have been discussed elsewhere (Waksman et al, 1933) in connection with other biological data secured at the same time. The water taken from the

Gulf of Maine proper, Stations 1329–1333, was, with a single exception, characterized by low numbers of bacteria similar to those found in the water off Cape Cod Light. The surface water at Station 1333 contained the exceptionally high number of 380 bacteria per cubic centimeter. No explanation for this is known except the possibility of the presence in the sample of the decaying remains of some small marine organism. The number of bacteria in the relatively shallow water over Georges Bank, Stations 1334–1336, was uniformly higher than in the Gulf of Maine. This paralleled a higher phytoplankton content in the Georges Bank water (Gran, 1933). The finding (Waksman et al, 1933) that large numbers of bacteria occur with the plankton, particularly the phytoplankton, suggests that the higher bacterial content of this water is due to increased available organic matter resulting from the high plankton content. Bacterial numbers did not vary significantly with depth at any of these stations. If the above explanation relating an increased bacterial content with an increased phytoplankton content is correct, then it would seem that the numbers of the two types of organisms should have a parallel variation with depth. The studies reported above were not detailed enough to determine whether or not this was the case. It is possible that with the low quantities of plankton ordinarily found in the sea during the summer months, it would be very difficult to detect variations of bacteria with depth during this time. A study of the numbers of bacteria associated with the annual spring increase of the plankton should afford results extremely interesting from the standpoint of the decomposition and transformation of the organic materials produced in lreatively large amounts at that time.

TABLE IV

Abundance of bacteria in sea water of Cape Cod Bay, August 24–September 21, 1932.

Station	Depth of water	Number of bacteria per cc. water			
		August 24		September 21	
		Surface water	Bottom water	Surface water	Bottom water
	meters				
1	26	8	23	—	—
2	28	19	15	—	—
3	35	17	26	—	—
4	38	9	36	2690	850
5	37	8	21	4670	720
6	26	26	23	2620	1020

Returning to the water of Cape Cod Bay, it will be seen that the number of bacteria found there on August 24 is significantly higher than that found on the same date off Cape Cod Light but approximately equal to the number occurring over Georges Bank on August 5. At three of these stations sampled on September 21 there was an increase in the number of bacteria over that found on August 24 amounting to practically a hundred-fold in both the surface and bottom water. This increase may or may not be due to the fact that the samples of September 21 were taken between 4:00 and 6:00 A.M., while those of August 24 were taken from 7:40 to 11:25 P.M. On August 24 the bottom water at four of the six stations contained more bacteria than did the surface water. Of the three stations sampled September 21, all contained from two to six times as many bacteria at the surface as at the bottom. The only factor under observation which varied at the two sampling periods was, as noted above, the time of day at which the samples were taken. This does not seem to offer an adequate explanation for the wide variation found in the distribution of the bacteria.

Abundance of Bacteria in Sea Bottom Deposits

The mud samples of which the bacteriological analysis is reported here were obtained at the same stations as those described above for the water samples. In general no attempt was made to sample the bottom deposit at those stations where it consisted only of sand. At a few stations, however, sand samples were obtained and these are designated as such wherever they occur in the tables. The deposits examined were entirely of terrigenous nature. The material from which the bacteria living in the bottom deposits of this type secure their food comes then from two sources: (1) The organisms living in or on the sea bottom and those sinking from the water above, (2) the organic matter present in a partially decomposed state in the sediments brought in from land. The relative amount of these two sources of organic matter will vary widely depending upon local conditions. The fresher organic matter tends to accumulate at the surface of the bottom and to become incorporated into the lower layers of mud only very slowly through the action of burrowing organisms living in the marine bottom. Sand bottoms contain relatively little organic matter, but the findings of Raymond and Stetson (1931) show that over them also there occurs a small amount of organic material.

The number of bacteria found in the ocean bottom at the stations south of Martha's Vineyard in July, 1931, is given in Table II. The figures for loss on ignition of the dry mud are also given as a rough indication of the amount of organic matter in the samples. The sand samples contained fewer bacteria than did the mud samples. This is especially true of the sand taken nearest shore where there is practically no organic matter present. In the mud samples there is a progressive diminution in the number of bacteria present with distance from shore. The number of bacteria in these samples cannot be definitely correlated with the amount of organic matter present, probably because in these very finely divided sediments a considerable part of the loss on ignition is due to the loss of physically or chemically bound water. The number of bacteria diminished rapidly with depth into the mud. Later determinations on mud samples from the Gulf of Maine indicated (Table V) the fact that they had a bacterial content of the same order of magnitude as the muds south of Martha's Vineyard. The figures for the number of bacteria in mud samples from Buzzards Bay and New Bedford Harbor given in Table I indicate the much higher bacterial content of sediments near land. The carbon content of these muds as determined by a modification (Tiurin, 1931) of the method of Schollenberger (1931) is given also. The determinations indicate a very close direct correlation between the number of bacteria in these samples and the content of organic matter as indicated by the carbon determination.

TABLE V

Abundance of bacteria in sea water and in bottom deposits of the Gulf of Maine and Georges Bank, August 2–5, 1932.

	Number of bacteria per cc. water							
Station No.	1329	1330	1331	1332	1333	1334	1335	1336
Depth of water in meters	256	207	230	346	230	71	74	64
Depth of sample								
Surface	10	3	4	2	380	16	92	6
50 meters	1	1	2	1	2	—	—	—
Bottom	6	0	1	1	3	25	41	27
Depth in mud	Number of bacteria per gram of dry mud or sand							
Surface 2.5 cm	5400	4700	830	3000	—	—	1280 (sand)	670 (sand)
10 cm	1720	1700	140	290	—	—	—	—

In the sampling method for mud, described above, the mud was obtained as a core in a glass tube. The core was removed from the tube by pouring off the water above the mud, inserting a small piece of sterile cotton, and then pushing the mud from the tube with the aid of a wooden rod slightly smaller in diameter than the inside diameter of the glass tube. By this procedure the surface layer of mud became somewhat compressed and mixed and the extreme surface was absorbed to some extent by the cotton. The surface sample of mud represented, therefore, a composite of approximately the top inch of mud. The organic matter added to the mud from the water above tends to accumulate at the very surface of the mud in a zone probably so thin as to be measured in millimeters rather than centimeters. A change in the procedure for removing the mud from the tube was then adopted in order to study the number of bacteria in the extreme surface layer of mud. In pouring off the water above the tube about 4 cc. were allowed to remain behind. Previous platings of this water had shown that unless mixed with mud very few bacteria were present. The tube was then shaken until the surface layers of mud were in suspension. One cubic centimeter portion of the suspension was then pipetted off for bacteriological analysis, another for moisture and total carbon determination, and another for a sea salt determination. The amount of dry material in one cubic centimeter of such a suspension was usually about 60 mgm., although it varied from 35 to 90 mgm. with different samples. Of the dry material, usually one-half or more, depending upon the concentration of mud in the suspension, was sea salt. The carbon and bacteriological determinations were corrected for the presence of this salt.

TABLE VI

Distribution of bacteria and organic carbon in the mud of the Atlantic Ocean off Cape Cod Light, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud							Per cent organic carbon in dry mud		
		August 24				September 20			September 20		
		Surface	2.5 cm.	10 cm.	20 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
6		5,000,000	630	340	—	125,500	4,300	270	5.20	1.30	1.37
7		919,000	1695	176	—	48,800	4,900	2,390	16.66	1.50	1.77
8		23,100	1490	220	0	25,300	4,700	2,290	5.77	1.72	1.77

The number of bacteria found in mud samples from Cape Cod Bay and the Atlantic Ocean off Cape Cod Light by the above method are given in Tables VI and VII. The carbon content of the mud is included in these tables. The carbon content of the surface layer was considerably higher than that of any of the lower layers. In two cases values as high as 16.66 and 20.00 per cent carbon in the surface mud were found, but these values may possibly be excessive. An inspection of this data shows at once the importance of the extreme surface layer of the mud from the standpoint of bacteriological activity. The number of bacteria in the surface exceeded by far the number present at a depth of only 2.5 cm. at all stations. Not only was the surface layer rich in total number of bacteria but it also contained a much more varied bacterial flora than did the lower layers. Agar plates of samples from the 2.5 cm. depth revealed only small, grayish white, either round or lens-shaped colonies. Plates from the surface layer, on the other hand, contained many types of colonies, including occasionally agar-decomposing forms. This surface layer of mud, having a high concentration of available organic matter and a source of available oxygen in the water above, appears to be the seat of the greatest activity of the marine bacteria. The number of bacteria in the surface mud varied markedly at the two sampling periods. However, the number of bacteria decreased on the ocean side of Cape Cod and increased correspondingly in the bay. It should be noted that, as shown in Table IV, the number of bacteria in the water of Cape Cod Bay also was markedly greater at this time than at the previous sampling period. It is generally assumed that there is an accumulation of sediment going on in Cape Cod Bay, part of this coming from the outside of Cape Cod. Whether there is any relation between this deposition of fresh sediment and the observed change in the bacterial content of the mud is uncertain. The fluctuation of the bacterial

TABLE VII

Distribution of bacteria and organic carbon in the mud of Cape Cod Bay, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud						Per cent organic carbon in dry mud		
		August 24			September 21			September 21		
		Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
1		43,600	9,560	3,200	—	—	—	—	—	—
2		76,000	9,680	2,000	—	—	—	—	—	—
3		217,000	11,000	—	—	—	—	—	—	—
4		236,500	16,000	7,200	2,700,000	32,200	28,800	4.50	1.77	1.90
5		134,600	24,300	9,500	1,160,000	54,700	47,700	7.50	2.46	2.85
6		248,000	44,300	15,500	8,420,000	79,300	60,000	20.00	3.10	2.76

content of the lower layers of mud is less marked. In Cape Cod Bay it was parallel with that of the surface mud, while off Cape Cod Light it was in the opposite direction. In the samples from the latter line there was again a progressive diminution in numbers of bacteria in the surface mud with distance from land. The explanation of this is not clear from the data available. In these samples the number of bacteria in the deeper layers did not decrease with distance from land. The vertical and horizontal distribution of bacteria in the mud of the Cape Cod Light line of stations is illustrated in Fig. 2. Because of the extremely wide range in the number of bacteria found, the logarithms of the numbers of bacteria are plotted, instead of the actual numbers themselves.

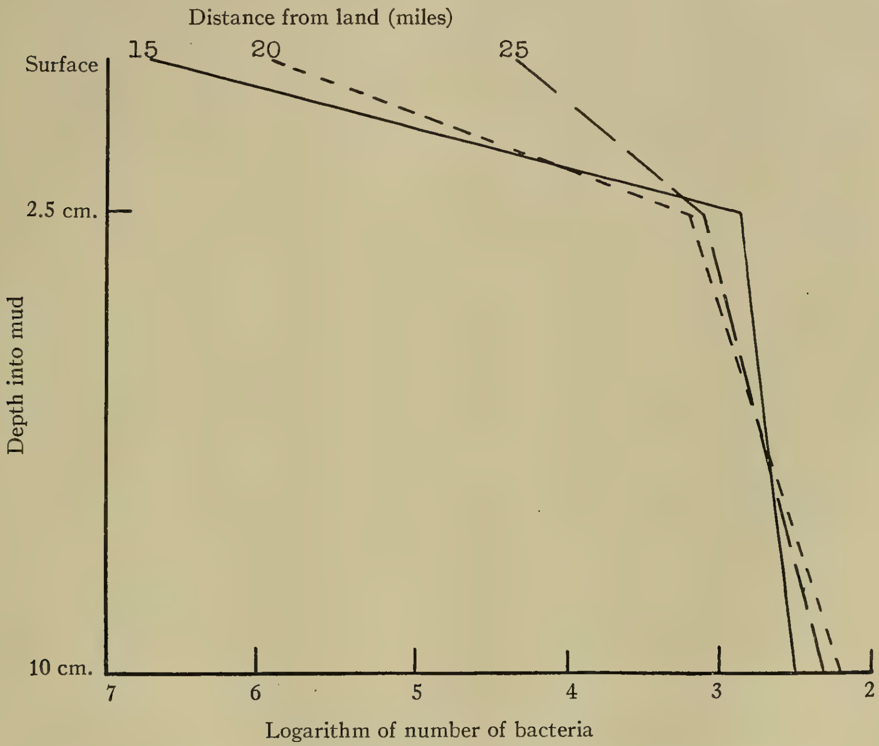

Fig. 2. Vertical and horizontal distribution of bacteria in the mud of the Atlantic Ocean off Cape Cod Light, August 24, 1932. —— Station 6; - - - Station 7; — — Station 8.

The relation between the bacterial content and the carbon content in the muds is very interesting. In the surface mud there does not appear to be a definite relation between the bacterial numbers and the amount of carbon. This is to be expected, since the number of bacteria in this layer is not only due to the abundance of organic matter, but perhaps to a greater extent to its relatively undecomposed nature as compared to that in the lower layers of mud. This dependence of the organisms upon the nature of the organic matter is illustrated also by the vertical distribution of bacteria and carbon; the number of bacteria invariably decreased with successive increases in depth in the mud, while the carbon content often rose slightly. Oceanic bottom deposits are built up principally by the accumulation of new material at the surface. The rate of deposition is extremely slow and consequently mud at relatively short depths into the mud represents sediments laid down many years before. The organic matter in the mud below the surface has undergone extensive decomposition and only the more resistant substances remain. These substances are probably similar to the resistant organic complexes in the soil described by Waksman and Iyer (1932) and are higher in carbon than is the fresh organic matter. These factors, together with the practical absence of oxygen from the mud (Moore, 1931), offer an adequate explanation for the decrease of bacterial numbers with depth in the mud. The resistant nature of the organic matter limits the number of specific organisms capable of attacking it. The high carbon and low oxygen content of the organic matter decrease its availability to anaerobic bacteria, while the absence of oxygen in the mud limits the activity of aerobic forms. At depths of 2.5 and 10 cm. in the mud the horizontal distribution of bacteria varied directly with the carbon content of the mud. This was especially true of the mud in Cape Cod Bay, where the variation in the two factors was so large as to lend greater weight to the observations. This would seem to indicate that in the terrigenous deposits examined the organic matter in the mud below the surface layer was equally available to the bacteria at all the stations sampled and probably was similar in composition. The factors determining the distribution of bacteria at the surface of the mud are still obscure. Undoubtedly an important one is the stage of decomposition of the organic matter at the time it reaches the bottom.

SUMMARY

A study has been made of the number of bacteria to be found in ocean waters and muds in the region of Cape Cod, Massachusetts, and their variation with distance from shore, depth of water, depth in the mud, and carbon content of the mud.

The number of bacteria in ocean water not near land and of a depth sufficient to preclude the agitation of the bottom by currents or wave motion was very small. One cubic centimeter portions of the water were often found to contain no bacteria capable of growing on an agar plate. A few samples of water were found to contain comparatively large numbers of bacteria, probably due to the presence of decomposing remains of some small plankton organism.

Sea water in shallow regions where the water was mixed from top to bottom, due to tidal or wave motions, had a consistently higher bacterial content, ranging from 20 to 200 cells per cubic centimeter. Such water had a higher content of mineral nutrients and consequently a richer plankton content. It is to the larger amount of organic matter thus made available to the bacteria together with an actual transport upward of cells by vertical currents that their increased abundance under these conditions is to be attributed.

On open, exposed shores receiving little land drainage, the direct effect of the land on the bacterial content of the sea water appears to be negligible. Along shores receiving considerable land drainage any effect of the proximity of land does not appear to extend beyond a mile from the shore so far as the number of bacteria in the sea is concerned. In harbors receiving sewage drainage, the number of bacteria may rise to very high levels, depending upon the amount of pollution introduced and the degree to which circulation may take place between the harbor water and the outer uncontaminated water.

Bacteria in the bottom deposits were much more numerous than in the overlying water. Muds contained many more bacteria than did sands. The number of bacteria decreased with depth into the mud, the most marked decrease being in the first 2.5 centimeters. Bacterial numbers in the surface layer, compared to those in the lower layers, were much greater than could be accounted for by the difference in organic carbon content. There appeared to be on the surface of the mud a very thin layer containing organic matter in a less advanced stage of decomposition supporting a bacterial growth much richer not only in numbers but also in types of bacteria than in the lower layers. The carbon content of the surface layer was higher than that of the lower layers. The vertical distribution of bacteria in the mud was not related to the carbon content, since the amount of carbon sometimes increased with depth, while the number of bacteria always decreased with depth.

The horizontal distribution of bacteria in the extreme surface layer of mud was independent of its carbon content, but probably depended upon its composition or the degree of its decomposition. The horizontal distribution of bacteria in the mud layers below the surface was directly related to their carbon content.

The number of bacteria in the surface mud on the continental shelf east of Cape Cod decreased regularly with distance from land. This was not the case with the bacteria in the deeper layers of mud, where the number remained fairly constant.

There was a wide variation in the bacterial content of all layers of mud between two sampling dates approximately one month apart. The fluctuations at the surface and at depths of 2.5 and 10 cm. in the mud were in opposite directions in mud from the open ocean off Cape Cod Light but were parallel in mud from Cape Cod Bay.

LITERATURE CITED

BAVENDAMM, W., 1932. Die mikrobiologische Kalkfällung in der tropischen See *Arch. Mikrobiol.*, **3**: 205.

BENECKE, W., 1933. Bakteriologie des Meeres. *Abderhalden's Handbuch der biologischen Arbeitsmethoden.* Abt. IX, Lfg. 404. S. 717–872.

BERKELEY, C., 1919. A Study of Marine Bacteria, Straits of Georgia, B. C. *Trans. Roy. Soc. Canada*, 3 ser., **13**: 15.

BUTKEWITSCH, W. S., 1932. Zur Methodik der bakteriologischen Meeresuntersuchungen und einige Angaben über die Verteilung der Bakterien im Wasser und in den Böden des Barents Meeres. *Trans. Oceanogr. Institute* (Russian), **2**: 37.

CASSEDEBAT, P. A., 1894. De l'action de l'eau de mer sur les microbes. *Rev. d'Hyg. et de pol. san.*, 1894, No. 2, p. 104. Abstr. *Centrbl. Bakt.*, Abt. I, **16**: 265.

CHOLODNY, N., 1929. Zur Methodik der quantitativen Erforschung des bakteriellen Planktons. *Centrbl. Bakt.*, Abt. II, **77**: 179.

DREW, G. H., 1913. On the Precipitation of Calcium Carbonate in the Sea by Marine Bacteria, and on the Action of Denitrifying Bacteria in Tropical and Temperate Seas. *Jour. Mar. Biol. Ass'n U. K.*, **9**: 479.

FISCHER, B., 1894a. Die Bakterien des Meeres. *Ergebnisse der Plankton Expedition der Humboldt-Stiftung*, **4**: 1.

FISCHER, B., 1894b. Die Bakterien des Meeres nach den Untersuchungen der Plankton expedition unter gleichzeitiger Berücksichtigung einiger älterer und neuerer Untersuchungen. *Centrbl. Bakt.*, **15**: 657.

FRANKLAND, E., AND W. T. BURGESS, 1897. Sea Water Microbes in High Latitudes. *Chem. News*, **75**: 1.

GAZERT, H., 1912. Untersuchungen über Meeresbakterien und ihren Einfluss auf den Stoffwechsel im Meere. *Deutsche Südpolar Expedition 1901–1903*, **7**: 235.

DE GIAXA, 1899. Über das Verhalten einiger pathogener Mikroorganismen im Meerwasser. *Zeitschr. Hyg.*, **6**: 162.

GRAN, H. H., 1933. Studies on the Biology and Chemistry of the Gulf of Maine. II. Distribution of phytoplankton in August, 1932. *Biol. Bull.*, **64**: 159.

LEVIN, 1899. Les microbes dans les régions arctiques. *Ann. l'Inst. Pasteur*, **13**: 558.

LIPMAN, C. B., 1929. Further Studies on Marine Bacteria with Special Reference to the Drew Hypothesis on $CaCO_3$ Precipitation in the Sea. *Carnegie Inst. Wash. Publ.* 391, pp. 231–248.

LLOYD, B., 1930. Bacteria of the Clyde Sea Area: a Quantitative Investigation. *Jour. Mar. Biol. Ass'n U. K.*, **16**: 879.

LLOYD, B., 1931. Muds of the Clyde Sea Area. II. Bacterial content. *Jour. Mar. Biol. Ass'n U. K.*, **17**: 751.

MOORE, H. B., 1931. The Muds of the Clyde Sea Area. III. Chemical and physical conditions; rate and nature of sedimentation; and fauna. *Jour. Mar. Biol. Ass'n U. K.*, **17**: 325.

MOORE, H. B., AND R. G. NEILL, 1930. An Instrument for Sampling Marine Muds. *Jour. Mar. Biol. Ass'n U. K.*, **16**: 589.

OTTO, M., AND R. O. NEUMANN, 1904. Über einige bakteriologische Wasseruntersuchungen in Atlantischen Ozean. *Centrbl. Bakt.*, Abt. II, **13**: 481.

PÜTTER, A., 1924. Die Atmung die Planktonbakterien. *Pflugers Arch. Physiol.*, **204**: 94.

RAKESTRAW, N. W., 1933. Studies on the Biology and Chemistry of the Gulf of Maine. I. Chemistry of the waters of the Gulf of Maine in August, 1932. *Biol. Bull.*, **64**: 149.

RASUMOW, A. S., 1932. Die direkte methode der Zahlung der Bakterien im Wasser und ihre Vergleichung mit der Kochschen Plattenkulture Methode. *Microbiology* (Russian), **1**: 145.

RAYMOND, P., AND H. C. STETSON, 1931. A New Factor in the Transportation and Distribution of Marine Sediments. *Science*, **73**: 105.

RUSSELL, H. L., 1892a. Untersuchungen über in Golf von Neapel lebende Bacterien. *Zeitschr. Hyg. Infk.*, **11**: 165.

RUSSELL, H. L., 1892b. Bacterial Investigation of the Sea and its Floor. *Bot. Gaz.*, **17**: 312.

RUSSELL, H. L., 1893. The Bacterial Flora of the Atlantic Ocean in the Vicinity of Woods Holl, Mass. *Bot. Gaz.*, **18**: 383, 411, 439.

SCHOKALSKY, J., 1928. L'Expédition océanographique de la mer Noire. *C. R. Acad. Sci. Paris*, **186**: 1707.

SCHOLLENBERGER, C. J., 1931. Determination of Soil Organic Matter. *Soil Sci.*, **31**: 483.

TIURIN, I. V., 1931. A New Modification of the Volumetric Method of Determining Soil Organic Matter by Means of Chromic Acid. *Pedology* (Russian), No. 5–6, pp. 36–47.

WAKSMAN, S. A., AND K. R. N. IYER, 1932. Contribution to Our Knowledge of the Chemical Nature and Origin of Humus. I. On the synthesis of the "humus nucleus." *Soil Sci.*, **34**: 43.

WAKSMAN, S. A., H. W. REUSZER, C. L. CAREY, M. HOTCHKISS, AND C. E. RENN, 1933. Studies on the Biology and Chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms. *Biol. Bull.*, **64**: 183.

WILSON, F. C., 1920. Description of an Apparatus for Obtaining Samples of Water at Different Depths for Bacteriological Analysis. *Jour. Bact.*, **5**: 103.

THE STRUCTURE AND DIVISION OF BODOPSIS GODBOLDI, SPEC. NOV.[1]

JAMES B. LACKEY

From the Department of Water and Sewage Research, N. J. Agricultural Experiment Station

Material and Methods

The organism for which the species name *godboldi* is proposed is a small flagellate protozoön of the order Pantastomatida (Calkins, 1926). The genus contains one other species, *alternans*, described by Lemmermann (1914). The writer is unaware of any cytological work or any descriptive work on the genus other than that of Lemmermann.

The form herein alluded to developed in large numbers from a culture of raw sewage from the Plainfield, New Jersey, sewage disposal plant. A 1 cc. sample of sewage was inoculated into a Petri dish containing 10 cc. of the wheat medium used frequently by the writer (Lackey, 1927). Out of ten cultures made up at the same time this organism appeared in but one, but several subcultures were made in which the animals multiplied abundantly. Each culture was about 5 mm. deep and had a film of paraffin oil on top so that as the bacteria and protozoa multiplied the medium was at least partially anaerobic. Neither percentage of dissolved oxygen nor pH were ascertained.

Cover-glasses with films containing the living organisms were sealed to slides with lanolin for studying the activities of the animals. Prepared slides were made by sinking cover-glasses in the cultures until the organisms crept onto them, then fixing the film with either Gilson's fluid or 95 per cent alcohol saturated with $HgCl_2$ to which .5 per cent acetic acid was added. The first fixative proved excellent for the pseudopodia, which are fine and tenuous, while the second was best for the study of mitosis. Iron hæmatoxylin was the stain most frequently used, occasionally followed by eosin.

Structure of the Animal

The living animal is generally ovate in outline and dorsiventrally bowed (Figs. 1, 2), but there is considerable deviation from this type shape. Individuals have a fixed shape, no amœboid changes having been observed. The average dimensions are 12 microns long, 6 wide, and 3 to 4 thick. This species is therefore slightly smaller than *B. alternans*. The pellicle is thin and unmarked. At the anterior end is a naked bowl-shaped depression, referred to as the mouth region. This is not at all like the gullet-reservoir system of the Euglenida. The two flagella emerge from this region and also the pseudopodia, when they are extended. Through the region food, including bacteria, is ingested. There is a contractile vacuole adjacent to this depression which empties into it. The flagella are dissimilar: the short one is about one-fourth body length, extends forward, and is vibratile. It is so short as to appear practically useless as far as locomotion is concerned. The longer is a trailing flagellum, half as long again as the body, and is held ventrally when the animal creeps or glides along the substratum.

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The animal moves forward very slowly, perhaps making use of a creeping movement as well as the traction of the short flagellum. Often it is motionless for a long time, then moves slightly with a jerky motion. It was this very slow and jerky motion of a very large number of small animals on the bottom of a Petri dish which first called the organism to the attention of the writer. The motionless periods represent the times when the pseudopodia are extended and the animal is probably feeding. It has not been observed swimming, which might be impossible with its very short flagellum. In *B. alternans* the two flagella appear to be of equal length and the difference in this respect is the most marked one between the two species.

The cytoplasm is hyaline and homogenous in appearance. Food vacuoles, at times containing bacteria and portions of small fungi as recognizable contents, and moving with a slow cyclosis, are often seen. In the posterior part of the cell a number of small bright spheres of unknown nature are usually present. The nuclear membrane with a clear zone just inside it and a heavy endosome are visible in the living animal.

The pseudopodia (Fig. 3) are best studied in fixed, overstained animals. From two to six in number, they emerge from the mouth region and extend laterally from the ventral surface as a rule. The cytoplasm at such times may flow out to partly cover an area of the pellicle, much as it extrudes from the mouth of the shell of an *Arcella*. The pseudopodia are very fine, of the filose type, and branch infrequently. Often there are small enlargements, blobs of protoplasm on them, and it has been definitely observed that they are used in the taking of bacteria. Where they come in contact or cross, they do not anastomose. Their greatest extension rarely exceeds twice body length. There is no axial filament.

The kinetic elements are a pair of blepharoplasts (Figs. 1, 4) adjacent to the nuclear membrane and in the thin layer of cytoplasm which is the floor of the mouth depression. It is very difficult to determine that there are two, so small and so close together are they. The nucleus is in the anterior part of the body and appears to be slightly pointed or pulled up to make contact with the blepharoplasts. Its membrane is rather heavy. There is always a central round endosome, with a small clear zone between it and the chromatin which instead of being in the form of granules appears to have the form of a permanent, faintly staining spireme. This detail is not to be ascertained with certainty in the interkinetic stages.

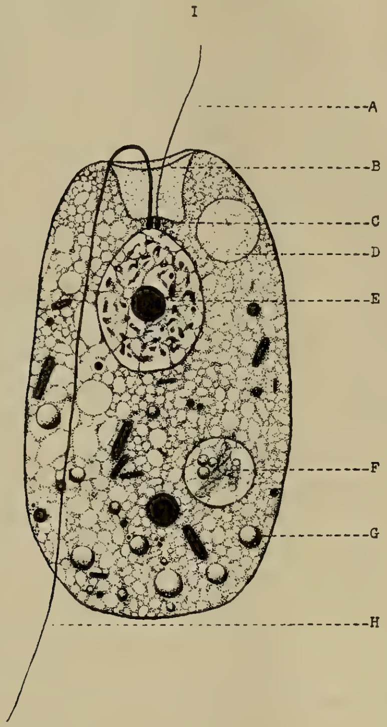

FIG. 1. *Bodopsis godboldi,* spec. nov. Greatly enlarged to show structure. Nuclear and kinetic structures drawn from prepared slide, cytoplasmic detail from a study of living animals. *A*—anteriorly directed flagellum. *B*—mouth depression. *C*—blepharoplasts. *D*—contractile vacuole. *E*—endosome, surrounded by the chromatin masses characteristic of the vegetative nucleus. *F*—food vacuole. *G*—cytoplasmic sphere, one of three common types of inclusions. Two others are shown, but not labeled: the black spheres, visible only after staining, and the black rods also visible only after staining. *H*—trailing or gliding flagellum.

CELL DIVISION

The animal apparently does not reach a maximum size before division, since both large and small ones are found dividing. Division is longitudinal in the active state, the nuclear membrane persisting throughout the process. The first indication of division is the increase in the staining capacity of the chromatin, which soon forms a number of heavy, irregular masses of chromatin, at first assumed to be comparable to the prochromosomes of some of the higher organisms (Fig. 4). Attempts to count the number of these have not been successful, partly because of their irregular contours and partly because of the presence of the endosome. They tend to assume a more or less peripheral position inside the nuclear membrane, and a number of counts have been made which show more than thirty of them. We have concluded after studying this particular phase of division that there are more than the metaphase number of chromosomes and less than twice the number, and that the number of these irregular chromatin masses is not constant.

As these masses are forming, the endosome begins to lose its power of taking the stain. It finally disappears altogether, simply becoming faint, then vanishing altogether, before the metaphase plate of chromosomes is formed. It does not contribute to the formation of blepharoplasts or new flagella, nor visibly to the formation of the chromosomes. It is possible that it is of chromatin as Belar (1920) has shown to be the condition relative to the endosome of *Prowazekia*.

By the time the endosome has disappeared, the chromosomes are fully formed. There are 24 of these and they are small round bodies, not connected with each other, and usually close to each other when they have oriented themselves on the intranuclear spindle. The origin of the spindle is impossible to trace; it first becomes visible at the time the metaphase plate of chromosomes is formed. Because of its small size it is impossible to distinguish spindle fibers, but the double cone shape and the orientation of the metaphase plate of chromosomes on it leaves no doubt as to its identity. There are no centrioles at its poles, but its ends reach to the nuclear membrane at this time (Fig. 6). It is impossible to say whether the ends of it still reach the membrane in the anaphase and telophase stages (Figs. 8, 9) or not, but spindle remains, presumably mantle fibers, are present in these stages. The spindle invariably occupies a position at right angles to the long axis of the cell, and the metaphase plate of chromosomes is therefore always in equatorial view, since animals are rarely found turned edgewise to the observer. For this reason it is difficult to find plates on which counts of the chromosome number can be made.

The chromosomes practically all divide at the same time, assuming a dumb-bell shape (Fig. 6*b*), then parting. There are none which lag on the spindle, and due to the fact that they are all approximately round in the beginning, no question of their plane of division can be discussed.

During these happenings the original pair of flagella has persisted,

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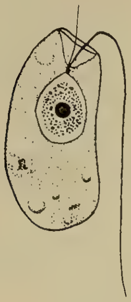

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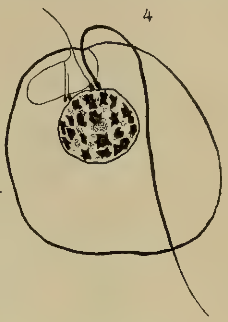

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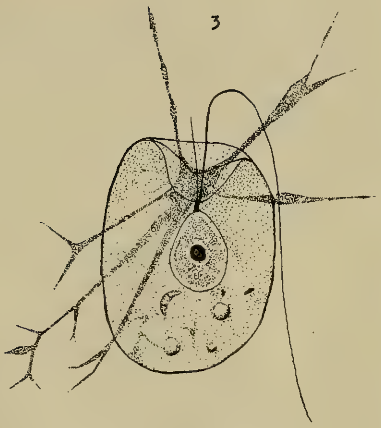

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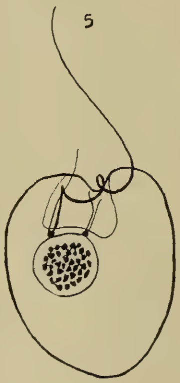

PLATE I

FIG. 2. Side view of the animal to show characteristic convex shape.

FIG. 3. Ventral view of an animal to show pseudopodia which are extended from the mass of cytoplasm extending out of and over the ventral lip of the mouth depression.

FIG. 4. Early prophase. The chromatin has formed into a number of irregular masses, apparently separate from each other and not forming a spireme. The endosome is disappearing. Two daughter blepharoplasts are present, and two new and short flagella are growing out from them through the mouth depression. This figure and the remaining ones are optical sections through the nucleus, but the flagella have been added to the sections.

FIG. 5. Later prophase. The endosome has disappeared and the chromatin masses are more condensed. The new blepharoplasts and flagella are clearly seen.

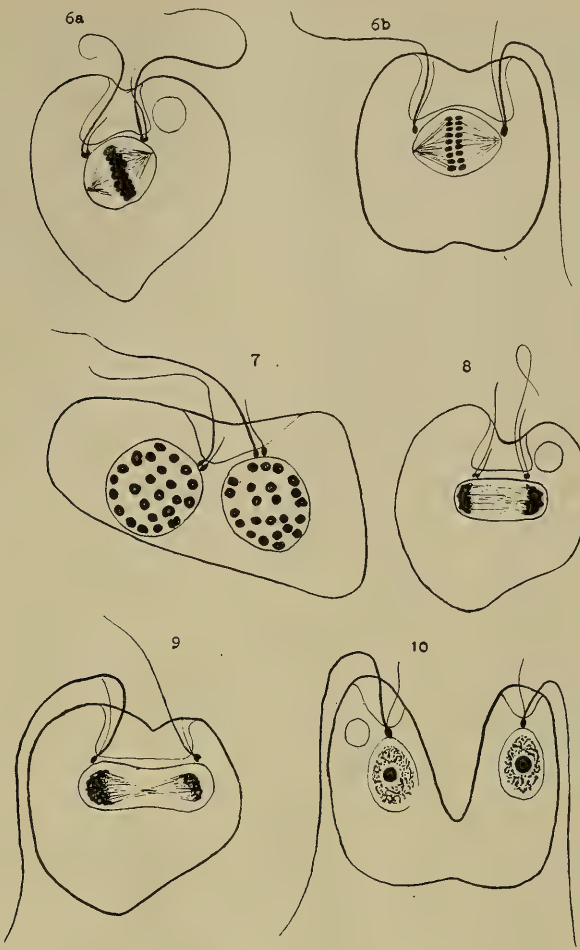

Plate II

Fig. 6*a*. Metaphase plate showing the equatorial mass of chromosomes, the intranuclear spindle, and the blepharoplasts and flagella.

Fig. 6*b*. Metaphase plate showing the dividing chromosomes.

Fig. 7. Anaphase. This figure is slightly diagrammatic in that the two groups of chromosomes have been actually shifted slightly to the right and left. In the animal studied they overlap each other about one-third but are so far apart that the two optical sections each give a clear count of the chromosomes. Each group consists of 24 chromosomes.

Fig. 8. Telophase. The chromosomes have reached the poles of the spindle. Clearly distinguishable spindle remains are seen.

Fig. 9. Telophase. Initial constriction of the cell and of the nucleus.

Fig. 10. Telophase. Nuclear reorganization practically complete and the two daughter cells about ready to separate.

but has come to occupy an eccentric position in the mouth depression which has widened. The blepharoplasts are near one pole of the mitotic figure, but do not form the pole (Fig. 6). During the prophases a second set of blepharoplasts appears at first close to the old ones, then moving apart from them toward the opposite pole of the spindle. It has been impossible to say certainly that this second pair of blepharoplasts arises from the original pair by division, but their position when first discernible, and their gradual migration away from the persistent original pair are strongly in favor of an origin by division, and against an origin *de novo*. It is certain that they do not arise from the endosome or any other nuclear component.

From this new pair of blepharoplasts, two new flagella grow out (Figs. 4 to 8 inclusive). The initial size difference between the two pairs is readily apparent, and precludes any question of the origin of new flagella by splitting: one of the daughter cells gets the old pair, the other the new pair. The animal is active throughout division.

In the anaphase the chromosomes move apart rapidly. Figure 7 shows polar views of the two daughter groups of chromosomes in which the number may be counted. The chromosomes become indistinguishably mingled in the telophase (Figs. 8 and 9) and still stain so deeply that it is impossible to tell when the endosome reappears. About this time a median anterior furrow begins to constrict the cell, dividing the mouth depression, and the nuclear membrane also begins to constrict, the cell finally pinching in two.

DISCUSSION

The Pantastomatida represent a very primitive group of protozoa and in the ten or twelve genera comprising this order we might reasonably expect to find primitive modes of reproduction. Such a condition has been described by Goldschmidt (1904, 1907) in the case of *Mastigella* and *Mastigina*, where the chromatin granules pass through the nuclear membrane and after accumulating on the outside, reform into the nuclei of gametes. Doflein (1916 *a* and *b*) has criticised the account of Goldschmidt, but has not shown him to be wrong. On the other hand, Minchin (1912) says that such a process would represent a step in the evolution of the protozoa from organisms such as bacterial types.

Accounts of division of other organisms of this order are lacking. It is therefore interesting to find in *Bodopsis*, representative of the order, a type of division not comparable to reproduction as described by Goldschmidt for *Mastigella* and *Mastigina* but more like that described by Wenyon (1926) for *Cercomonas*, Hartmann and Chagas

(1910) for *Spongomonas*, Belar (1920, 1921) for *Bodo edax* and *Collodictyon*, or even some of the Phytomonadida as shown by Aragao (1910), Doflein (1916, *a* and *b*) and Kater (1925). The pseudopodia of *Bodopsis* clearly place it in the Pantastomatida, but its type of nuclear division brings it into a more closely conformed relationship with these protozoa of related orders. Gamete formation in the manner described by Goldschmidt is not precluded, however, since gamete formation might occur at some stage in the life history not covered by this account.

It is noted that the nuclear membrane is persistent throughout division. This is not new, as shown in *Cercomonas*, *Spongomonas*, and *Cyathomonas* by Hartmann and Chagas, *Polytoma* by Kater, *Peranema* and *Entosiphon* by Lackey (1929, 1933), and *Euglena* by Baker (1926) and others, as well as various other protozoa. In *Bodopsis*, however, the chromatin lies just beneath the nuclear membrane as if the nucleus is turgid with a fluid pressing the masses of chromatin against the membrane. But no diffusion out or passage out of chromatic material can be noted. Instead the whole mechanism of chromosome formation and division is intranuclear even including the spindle. It is a fascinating idea that the endosome might give rise to the spindle. Thus in *Bodopsis*, *Cercomonas*, *Spongomonas*, *Cyathomonas* and other protozoa where there is an endosome which completely disappears during division, a well-defined spindle is formed which is an intranuclear structure in the four above-listed forms. On the other hand, in *Entosiphon*, *Peranema*, *Euglena* and others where the endosome does not disappear during division, no spindle is formed.

A peculiarity of *Bodopsis* is that while no centrioles are present, the blepharoplasts do not serve as division centers. It has been repeatedly stated recently that it is a difficult matter to determine just what is a centriole, but certainly there are no distinguishable ones in *Bodopsis*, whereas in most of the flagellates whose division has been carefully studied, either centrioles or blepharoplasts serve as poles of the division figure, the relations between these having been admirably diagrammed by Minchin (1912, p. 88). Centrioles have been figured for *Polytomella*, *Polytoma*, *Collodictyon*, *Bodo*, *Spongomonas splendida* and *S. uvella*, *Astasia* and others. Blepharoplasts serve as division centers in *Cercomonas*, *Peranema*, *Entosiphon*, and *Menoidium* (Hall, 1923). Since neither of these serves as a division center in *Bodopsis*, the question arises as to what actually constitutes the division center; or if there is one. The apparent absence of one is a condition unique for the flagellate protozoa.

If the primitive living cell has a distributed nucleus, it is hardly to be expected that the flagella of such a cell will possess blepharoplasts and the writer knows of no mention of them by bacteriologists. On the other hand, the possession of blepharoplasts by *Bodopsis* in juxtaposition to a well-organized nucleus, even if they do not serve as division centers, would argue that it is a specialized, not a primitive organism. This is further borne out by its mitotic type of nuclear division. And unless there is some other stage in its life history in which phenomena occur more comparable to those described by Goldschmidt for *Mastigina*, it is perhaps wiser to base the concept of the primitive condition of the order on the possession of both flagella and an amœboid body with pseudopodia.

Conclusions

1. *Bodopsis godboldi*, spec. nov., is shown to have a very short anteriorly directed flagellum, this being the chief character in which the species differs from *B. alternans*.

2. Its nuclear division is accomplished in the active state and is followed by binary fission.

3. The endosome disappears and the chromatin forms 24 small round chromosomes on an intranuclear spindle which has neither centrioles nor blepharoplasts for poles.

4. The original blepharoplasts and flagella are retained. New blepharoplasts apparently arise from the old ones by division, and new flagella from the new blepharoplasts by outgrowth.

5. The blepharoplasts occupy eccentric positions on the nuclear membrane while mitosis is occurring but do not form the spindle poles. It is concluded they are not the division centers.

REFERENCES

Aragao, H. de B., 1910. Untersuchungen über Polytomella agilis, n. g., n. sp. *Mem. Inst. Osw. Cruz.*, **2**: 42.

Baker, W. B., 1926. Studies in the Life History of Euglena. *Biol. Bull.*, **51**: 321.

Belar, K., 1916. Protozoenstudien. I. *Arch. f. Protist.*, **36**: 13.

Belar, K., 1920. Die Kernteilung von Prowazekia. *Arch. f. Protist.*, **41**: 308.

Belar, K., 1921. Protozoenstudien. III. *Arch. f. Protist.*, **43**: 431.

Berliner, E., 1909. Flagellaten-Studien. *Arch. f. Protist.*, **15**: 297.

Calkins, Gary N., 1926. Biology of the Protozoa. Lea and Febiger, Philadelphia.

Doflein, F., 1916a. Lehrbuch der Protozoen, 4th ed. Gustav Fischer, Jena.

Doflein, F., 1916b. Polytomella agilis. *Zool. Anzeig.*, **47**: 273.

Goldschmidt, R., 1904. Die Chromidien der Protozoen. *Arch. f. Protist.*, **5**: 126.

Goldschmidt, R., 1907. Lebensgeschichte der Mastigamöben Mastigella vitrea n. sp. und Mastigina setosa n. sp. *Arch. f. Protist.*, Supplement 1.

Hall, R. P., 1923. Morphology and Binary Fission of Menoidium incurvum (Fres.) Klebs. *Univ. Calif. Pub. Zoöl.*, **20**: 447.

Hartmann, M., and C. Chagas, 1910. Flagellatenstudien. *Mem. Inst. Osw. Cruz.*, **2**: 64.

Kater, J. McA., 1925. Morphology and Life History of Polytomella citri, n. sp. *Biol. Bull.*, **49**: 213.

Lackey, James B., 1927. A Culture Medium for Free-living Flagellates. *Science*, **65**: 261.

Lackey, James B., 1929. Studies in the Life Histories of Euglenida. I. *Arch. f. Protist.*, **66**: 177.

Lackey, James B., 1933. Studies in the Life Histories of Euglenida. III. *Biol. Bull.*, **65**: 238.

Lemmermann, E., 1914. Heft 1, Flagellatae 1. *In* Die Süsswasserflora Deutschlands, Österreich und der Schweiz *by* A. Pascher and E. Lemmermann. Gustav Fischer, Jena.

Minchin, E. A., 1912. Introduction to the Study of the Protozoa. Edward Arnold, London.

Wenyon, C. M., 1926. Protozoölogy. William Wood and Co., New York.

ON A NEW GREGARINE FROM THE CŒLOM OF A BURMESE EARTHWORM, PHERETIMA COMPTA

G. E. GATES

FROM JUDSON COLLEGE

In certain oriental species of earthworms, especially of the genera *Eutyphœus* and *Pheretima*, gregarines (trophozoite stages) are very frequently found in the cœlom. These gregarines when present may occur in large numbers, 50–90 per segment for a number of segments. Among the forms that have come under observation during the course of a study of Burmese earthworms is one which appears to be of especial interest and which is described below under the designation *Nellocystis birmanica, n.g., n. sp.*

Hitherto it has been assumed that the effect of the gregarine parasites on their earthworm hosts is not serious. Indeed, Stephenson in his monograph on the Oligochætes says, "The intestinal and cœlomic parasites have scarcely any effect on the tissues of the host." There is, however, some evidence to show that cœlomic sporozoa may have a drastic effect on host earthworms. A part of this evidence is included in a paper on the systematics of the Burmese earthworms that will appear shortly in the Records of the Indian Museum. It is hoped that the rather fragmentary observations contained in the paper will serve to indicate to protozoölogists a number of interesting opportunities for investigation.

NELLOCYSTIS BIRMANICA, N. G., N. SP.

The parasites are present as solitary individuals or aggregates of two or three individuals.

Solitary Individuals

The exact length of the parasites was not determined as the individuals were broken in removing them from the host for study. The form may be described as club-shaped, the posterior end enlarged, narrowing to an elongate stalk. The anterior end of the stalk is branched into several short filaments which attach the parasite to the host. In Fig. 2 is shown a portion of the anterior end of a parasite as it appears after being pulled out with forceps from a very delicate intersegmental septum.

In the fixed condition the stalk and a thin layer of the posterior end is solid and can be cut up into fragments which do not lose their shape.

Within the posterior portion of the parasite is a cavity, referred to as the vacuolar cavity, containing a fluid in which there is a single, dark, regularly spheroidal mass. The mass nearly fills the cavity and when ruptured in water or alcohol separates into granules which are connected with each other by fine cobwebby strands. Within the granular mass is a single, ovoidal nucleus. This is of a clear, transparent blueish or yellowish appearance and contains a single endosome. Within the stalk near the vacuolar cavity is a second nucleus which is like the vacuolar nucleus but which contains in addition to a large endosome, a smaller, slightly bent, rod-like body. In some of the indi-

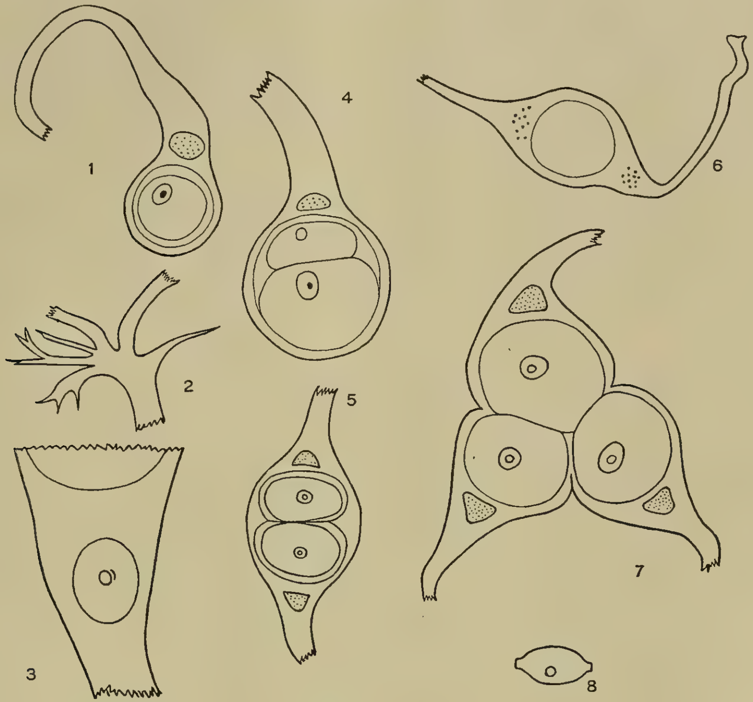

FIG. 1. Portion of a solitary individual, × 50.
FIG. 2. Portion of an anterior end of a solitary individual, × 160.
FIG. 3. Portion of a solitary individual showing the stalk nucleus in longitudinal position, × 160.
FIG. 4. Posterior end of a solitary individual containing two vacuolar masses, × 50.
FIG. 5. Central portion of a bi-stalked form with vacuolar septum, × 50.
FIG. 6. Bi-stalked form in which the vacuolar cavity was filled with cysts, × 50.
FIG. 7. Tri-stalked form, × 50.
FIG. 8. Cyst, × 330.

viduals the long axis of the stalk nucleus is along the long axis of the stalk (Fig. 3). In other individuals the major axis of the stalk nucleus is transverse to the long axis of the stalk (Fig. 1). In all those specimens in which the stalk nucleus is in the transverse position the single, large endosome is replaced by a number of smaller, endosomal granules scattered throughout the nucleus.

Several individuals were found in which the single vacuolar cavity contains two discrete, ovoidal masses, in each case one of the masses larger than the other. Each of these two masses has a single nucleus with an endosome. In specimens with two vacuolar masses the stalk nucleus is not ovoidal but the side of the nucleus towards the vacuolar cavity is flattened as shown in the figure. The endosomal material of the stalk nuclei is in the form of granules scattered through the nucleus. A few individuals differ from those just described in the replacement of the single nucleus of each vacuolar mass by several smaller bodies apparently of a nuclear nature.

Aggregates of Two or Three Individuals

Bi-stalked Forms.—These forms are spindle-shaped and have a stalk at each end so that the animal is doubly attached to the host. Stalks of all of these were broken in the course of dissection of the worm or in removing the parasites and the ends were not recovered so that it is not known whether the attaching ends have branched filaments similar to those at the anterior ends of solitary individuals. In some of these bi-stalked forms the vacuolar cavity is divided into two portions by a thin but distinct septum, each half of the cavity containing a single, dark, ovoidal mass. Each of the vacuolar masses has a single nucleus with an endosome. There are two stalk nuclei, one at each side of the partitioned vacuolar cavity, the side of the nucleus towards the cavity always flattened. In other bi-stalked forms the vacuolar septum is lacking and within the vacuolar cavity there is a single, dark mass. No nucleus is at first visible within the mass but if the mass is ruptured and flattened out carefully there may be seen among the granules small, ovoidal bodies similar in appearance to an endosome but smaller and with a cobwebby material attached to the surface so that each has a rather fuzzy appearance. In some specimens the number of these bodies is rather small; varying apparently from 16–32. In other specimens the number is much larger.

In a few bi-stalked forms the vacuolar cavity is filled with cysts. No definite stalk nuclei were observed in cyst-containing forms but at the approximate site of each nucleus there is an aggregation of endosomal-like granules. The regions between and around the endosomal granules are occupied by stalk substance rather than the clear and transparent extra-endosomal nuclear material.

The cysts are pseudonavicella-like (homopolar). There is a single nucleus, eccentrically located within the finely granular contents. One of the cysts is shown in Fig. 8.

Tri-stalked Forms.—A number of tri-stalked forms were found, aggregates of three individuals, each individual with a nucleated vacuolar mass and a stalk nucleus flattened on the vacuolar side and containing numerous endosomal granules. Attaching structures at ends of the stalks were not found.

Occurrence

Attached to the cœlomic face of the body wall, to the gut, and to the septa, in the region of segments viii–xxv of the type specimen of *P. compta* Gates 1932. The host was secured in the jungle near Blachi on the Karen Hills (western border of the Shan Plateau) to the east of Toungoo, Province of Burma, in the month of September.

Remarks

Although the host specimen was collected and preserved under unfavourable conditions the parasites are well preserved so that the various structures can be easily made out without either clearing or staining, the specimens having been examined as a rule only in the fluid used for preservation of the host. The figures are camera lucida sketches made immediately after removal of the parasites and prior to staining and clearing.

SUMMARY

A new species of gregarine protozoan, *Nellocystis birmanica*, elongately club-shaped, attached to the host by short branches of the anterior end, with two nuclei, parasitic in the cœlom of an earthworm, *P. compta*, from the Toungoo Hills of Burma, is described.

REFERENCES

Gates, G. E., 1933. Earthworms of Burma. IV. Records of the Indian Museum. (In press.)

Stephenson, J., 1930. The Oligochæta. London.

OSMOTIC PROPERTIES OF THE ERYTHROCYTE

VI. The Influence of the Escape of Salts on Hemolysis by Hypotonic Solutions

M. H. JACOBS AND ARTHUR K. PARPART

(From the Department of Physiology, University of Pennsylvania, the Department of Biology, Princeton University, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

I

In its osmotic behavior the erythrocyte shows certain anomalies that have never been completely explained. For example, it is known to undergo considerably less swelling in hypotonic solutions than it would if it were a perfect osmometer. This is particularly true of hypotonic solutions of non-electrolytes. A part of the discrepancy between the observed and the theoretical behavior of the cell is undoubtedly due to the fairly large fraction of its volume occupied by hemoglobin, and, to a much less extent, by other protein molecules; indeed, according to Ege (1922), when an entirely reasonable allowance of 35 or 40 per cent of the cell volume is made for this "osmotic dead space," the discrepancy largely vanishes. In the opinion of other workers, however (Gough, 1924; Krevisky, 1930; Ponder and Saslow, 1930), the difference between the observed and the theoretical volume changes is too great to be accounted for in this way; according to them the apparent dead space may amount to as much as 65 or 70 per cent of the total volume. Whatever may be the truth about this disputed point, it is certain that some other factor must be involved in producing the exceptionally low volumes observed in non-electrolyte solutions.

Two chief explanations have been offered to account for the above-mentioned peculiarities of the erythrocyte. The first (Gough, 1924; Krevisky, 1930; Ponder and Saslow, 1930) is that a part of the water in the cell is "bound" in such a way that it is unable to take part in osmotic exchanges. This explanation is not in accord with the results of Hill (1930), whose vapor pressure measurements indicate that no considerable part of the water of the erythrocyte fails to make its proper contribution to the colligative properties of the cellular solutions. For this reason, Ponder and his collaborators (Ponder and Saslow, 1931; Macleod and Ponder, 1933) have more recently strongly supported a second explanation, namely, that the failure of the erythrocyte to swell to the expected extent is due to a rapid escape of osmotically active materials from the cell into such solutions, particularly into those of non-electrolytes. In favor of this theory, in addition to their own important evidence, they cite the demonstration by Kerr (1929) that the erythrocytes of several mammals lose their normal impermeability to cations in protein-free solutions. The work of Joel (1915) on the progressive increase in the electrical conductance of suspensions of erythrocytes in solutions of sucrose is probably also to be interpreted in the same way, and numerous other observations scattered through the literature add further evidence in the same direction.

In view of all the available information, it can scarcely be doubted that under appropriate conditions an escape of salts from the erythrocyte into various non-physiological solutions takes place; and such an escape may well account for some of the osmotic peculiarities of this type of cell. Additional evidence of its occurrence will be given in the present paper. We believe, however, as a result of the observations about to be described, and of others as yet unpublished, that in experiments of short duration a leakage of osmotically active materials from the erythrocyte is a factor of relatively minor importance. In particular, we believe that the peculiar osmotic behavior of the erythrocyte in solutions of non-electrolytes, which has been the cause of much speculation since it was first observed by Hamburger, can to a considerable extent be accounted for in an entirely different manner, without postulating any loss of the normal impermeability of the cell to cations and without invoking factors other than those generally agreed to operate under typical physiological conditions.

II

The method we have employed is based upon a very briefly recorded observation of Green (1925), who reported that human erythrocytes, which ordinarily always undergo hemolysis in a 0.3 per cent solution of NaCl, may fail to do so after a preliminary treatment with non-hemolytic hypotonic solutions. The most plausible explanation of this behavior, which is the one proposed by Green, is that during the initial exposure to the non-hemolytic solution the swollen cells lose salts, and having thus acquired a lower internal osmotic pressure, they swell less than would otherwise be expected when the external concentration is further lowered. In this way they may escape hemolysis in hypotonic solutions in which it would ordinarily occur.

Unfortunately Green mentions no times in his account of his experiments, so it is impossible to be certain from his description whether the phenomenon he observed occurs rapidly or slowly. Furthermore, his method of comparing the osmotic resistance of the treated and the untreated cells is a very rough one. It seemed advisable, therefore, to take up the problem again in a more accurately quantitative manner. This we have done, employing the method described in the first paper of this series (Jacobs, 1930). By its use it is possible to measure percentages of hemolysis with a highly satisfactory degree of reproducibility, provided that the following precautions be used: (1) accurate measurement of the blood, (2) maintenance of a constant temperature, (3) maintenance of a constant pH, and (4) slow but constant stirring throughout the experiment. These precautions we have observed. In particular the pH of all our NaCl solutions was adjusted in the manner more fully described in the second paper of the series (Jacobs and Parpart, 1931) to approximately the normal blood reaction, and the temperatures were kept within a few tenths of a degree of those recorded in the tables.

TABLE I

Ox erythrocytes exposed for varying times to 0.110 M NaCl and then allowed to undergo hemolysis in 0.094 M NaCl. Temperature, 19.8° C.

Time of exposure	Final percentage of hemolysis
0	69
1 minute	69
10 minutes	69
30 minutes	68
1 hour	69
3 hours	68
5 hours	65
7 hours	62
9 hours	59
11 hours	56
18½ hours	54
21 hours	49

The general procedure followed in all the experiments may be made clear by outlining a typical one whose results are summarized in Table I. In this experiment, which was performed upon ox blood, preliminary determinations showed that 69 per cent of the erythrocytes underwent hemolysis in an hour in a 0.094 M solution of NaCl. This percentage showed little or no increase on further exposure, and could be obtained accurately and reproducibly in successive experiments on the same lot of blood. Different samples of blood were therefore exposed to a slightly more concentrated, non-hemolytic, but still strongly hypotonic (0.110 M) solution of NaCl and then, after times varying from one second to many hours, the concentration of this solution was suddenly reduced by dilution to 0.094 M. In order that hemolysis might, in all cases, take place with constant proportions of blood and of the hemolytic solution, the standard degree of hemolysis was determined by adding two drops of blood to 50 cc. of 0.094 M NaCl, the initial exposure to 0.110 M NaCl was made by adding two drops to 25 cc. of solution, and the subsequent dilution was brought about by mixing with this suspension an equal volume of 0.078 M NaCl. In this way the proportions of blood and solution were kept exactly the same during the process of hemolysis in all the experiments and in the control, namely, two drops of blood to 50 cc. of solution.

It would be expected that if an escape of salts occurred in the manner postulated by Green during the preliminary exposure to the nonhemolytic hypotonic solution, a lower degree of hemolysis would be found after this treatment than in the control. Since the method of measurement we employed is a delicate one, giving distinct differences in hemolysis with osmotic pressure, differences of a fraction of one per cent of that of blood plasma, any considerable loss of salts could scarcely have escaped detection. Nevertheless, as seen in Table I, the expected increase in the osmotic resistance of the cells began to be visible only after 3 to 5 hours, and its subsequent progress, though steady, was decidedly slow. The same general result was obtained in seven successive experiments with ox blood without a single exception. It seems likely, therefore, that with this particular kind of blood, which was used almost exclusively in our earlier work on osmotic hemolysis, an escape of salts in the course of experiments whose individual duration was usually from a few seconds to an hour at most could scarcely have been a disturbing factor of much significance. The criticism of this work by Macleod and Ponder (1933) on the grounds that we neglected the escape of salts from our cells, though theoretically an entirely valid one, would seem practically to be no more serious than similar criticisms that might be made of any of the simplifying assumptions that entered into our admittedly preliminary and approximate calculations.

It might perhaps be thought that in the experiment just described the initial degree of swelling of the cells was insufficient to permit a ready escape of salts but that a greater degree might have done so. To meet this objection, experiments were carried out in which a preliminary exposure was made to a solution which caused a considerable amount of hemolysis. In this case, the cells that just escaped hemolysis might be expected to show the optimum conditions for the escape of salts; nevertheless, although our method was capable of detecting osmotic changes of the expected sort in any group of more than one or two per cent of the cells used, such changes consistently failed to appear with ox erythrocytes in times less than several hours. We may conclude, therefore, that even under the most extreme conditions of swelling possible without hemolysis, the escape of salts from these cells must be fairly slow.

Another possible objection is that perhaps the escape of salts occurs so rapidly that even in the control it is complete before hemolysis has had time to take place, and for this reason differences between the experiment and the control fail to appear. In answer to this objection, which evidently cannot be completely met by experiments on hemolysis alone, it may be said that any escape of materials from the erythrocyte that proceeds as rapidly to a fixed end-point as the one just postulated can scarcely be the same as those studied by Kerr and by Joel, which apparently take place gradually and progressively over a period of hours. It is also difficult to believe that it is the same as the one indicated after 5 hours in Table I, since it is highly unlikely that a mere diffusion process would proceed almost instantly to some fixed point, then cease for several hours, only to be resumed again at the end of that time. If a rapid, indeed apparently an almost instantaneous, change in the osmotic properties of the erythrocyte occurs on placing it in a hypotonic solution—which is by no means inconceivable—it is probably due to some other cause than a mere outward diffusion of salts. The extreme definiteness of the behavior of erythrocytes in hypotonic solutions, which is brought out very clearly by the work of Ponder and Saslow (1931), suggests the possibility of a changed ionic equilibrium of some sort rather than a mere leakage of salts. In the case of non-electrolyte solutions, we believe that a plausible suggestion can be made as to the possible nature of such an ionic equilibrium, and this will be given below; in the case of electrolyte solutions, however, we have at present no definite explanation to offer.

III

The experiments so far described were performed upon the erythrocytes of the ox, which had been used in most of our previously published work. After confirming our earlier impression that the hemolytic behavior of these cells is probably not complicated to a troublesome extent in experiments of moderate duration by a leakage of salts, we examined in the same way the erythrocytes of several other species of mammals. The result of these experiments was to show specific differences of a rather surprising magnitude. Unlike the erythrocytes of the ox, which remained apparently unchanged in non-hemolytic hypotonic solutions for a number of hours, those of the pig, cat, rabbit and, in the single experiment as yet made, those of man usually showed fairly marked changes within an hour, and in some cases within a half-hour or less. A typical experiment with erythrocytes of the cat is summarized in Table II, and one with those of the rabbit in Table III. In the case of the cat cells, it will be noted that a decided increase in resistance was apparent in 30 minutes, and that roughly as much change had occurred in 3 hours as had been previously found with the ox cells in 21 hours. The erythrocytes of the pig seem to be somewhat more variable in their properties than those of the cat, the observed differences apparently depending to a considerable extent on the "age" of the cells. At the one extreme was a case where a marked increase in resistance occurred in 10 minutes and, at the other, one where no change was apparent in 2 hours.

TABLE II

Cat erythrocytes exposed for varying times to 0.100 M NaCl and then allowed to undergo hemolysis in 0.087 M NaCl. Temperature, 21.2° C.

Time of exposure	Final percentage of hemolysis
0	76
1 minute	75
10 minutes	74
30 minutes	70
50 minutes	69
70 minutes	68
90 minutes	66
2 hours	65
3 hours	59
4½ hours	59
7 hours	53

TABLE III

Rabbit erythrocytes exposed for varying times to 0.095 M NaCl and then allowed to undergo hemolysis in 0.072 M NaCl. Temperature, 22.7° C.

Time of exposure	Final percentage of hemolysis
0	58
1 minute	57
5 minutes	61
10 minutes	62
30 minutes	59
1 hour	51
2 hours	45
3 hours	36
4 hours	30
5 hours	28
8 hours	29

In the experiments on rabbit erythrocytes, a typical example of which is represented in Table III, the results were somewhat unexpected. The fall in the percentage of hemolysis which we have interpreted as being due to an escape of salts occurred fairly rapidly, as with the cat, but unlike the cases previously described, this increase in resistance was preceded by a temporary fall. In other words, rabbit cells after short exposures to a non-hemolytic hypotonic solution seem to be slightly more easily hemolyzed than the controls, though after longer exposures they behave in the more typical reverse manner. Perhaps factors such as those discussed by Ege (1921) may be involved. In connection with the use of rabbit erythrocytes, for osmotic experiments, reference may also be made to the recent work of Csapó and Kerpel-Fronius (1933), who have pointed out that this species is remarkable among mammals in the large fluctuations it shows in the depression of the freezing-point of its blood. According to these authors the rabbit is able to maintain constancy of blood pH only at the expense of the constancy of osmotic pressure usually found in mammals. For this reason it would seem that until the properties of rabbit's blood have been very fully investigated, considerable caution should be used in extending to other species conclusions drawn from experiments on it alone.

From this brief summary of our results on the erythrocytes of several species of mammals other than the ox, it is evident that not all kinds of blood are equally suitable for osmotic experiments. To the extent that changes such as those indicated in Tables II and III occur, the escape of salts from swollen cells must be regarded as a disturbing factor in all such experiments. The magnitude of the resulting errors, however, is usually not so great as might be imagined, since the method here employed is a delicate one which brings out clearly small differences. For example, a change in the percentage of hemolysis from 76 per cent to 70 per cent, such as occurred in the erythrocytes of the cat in 30 minutes, corresponds to a concentration difference of the order of magnitude of 0.001 M NaCl, *i.e.*, to an osmotic pressure difference less than 1 per cent of that of the blood. While, therefore, the erythrocytes of the pig, the cat, and the rabbit are obviously less suitable for osmotic experiments than are those of the ox, which by a lucky accident we happen to have used in most of our previous work, even they may be expected in experiments of not too great duration to yield results that are not hopelessly complicated by an escape of salts.

IV

The apparently abnormal osmotic behavior of erythrocytes in solutions of non-electrolytes has been recognized since the early work of Hamburger (for a general summary, see Hamburger, 1902). The view that this behavior is due to an escape of salts was expressed by Bang

(1909) and has recently received additional support from Ponder and Saslow (1931). Our own experiments on hemolysis in hypotonic non-electrolyte solutions, made by the method already described, confirm the general conclusion that osmotically active substances escape with especial readiness into such solutions. They indicate, however, that there is an earlier and a more characteristic effect of non-electrolyte solutions of a very different nature, which is entirely compatible with, and indeed dependent on, the normal impermeability of the erythrocyte to cations. The escape of salts, *i.e.*, of both anions and cations, which undoubtedly occurs, seems to be a later and a more pathological phenomenon.

TABLE IV

Ox erythrocytes exposed for varying times to 0.2 M sucrose and then allowed to undergo hemolysis in 0.125 M sucrose. Temperature 20° C.

Time of exposure	Final percentage of hemolysis
0	61
1 second	56
5 seconds	52
10 seconds	35
20 seconds	24
30 seconds	23.5
1 minute	21.5
5 minutes	18.5
10 minutes	18.0
15 minutes	18.0
20 minutes	13.0
30 minutes	7.0
45 minutes	5.5
60 minutes	0

In Table IV are presented the results of a typical experiment on ox erythrocytes with a non-electrolyte solution. In striking contrast with the behavior of these cells in hypotonic non-hemolytic NaCl solutions in which their normal osmotic properties are maintained for hours is that in similar solutions of sucrose. In the present experiment it will be noted that as much change occurred in 1 second as in that previously described (Table I) in perhaps 5 hours. Within 20 seconds the change was greater than that ever obtained in a day with a hypotonic NaCl solution. Do such results indicate an equally great difference in the rate of escape of salts from the cells in the two solutions? We believe that they do not, but that the change that occurs in erythrocytes exposed to non-electrolyte solutions consists of two phases: a very rapid ionic shift, whose effects are apparent within a few seconds, and complete within a minute or two, and following this, usually after a distinct pause, which in the present experiment amounted to approximately 10 or 15 minutes, a much slower leakage of salts from the cell, which is, nevertheless, considerably more rapid, and which begins much earlier than is the case with the similar change in electrolyte solutions (cf. Table I). As far as the second change is concerned, we are in agreement with Bang (1909) and with Ponder and Saslow (1931). We differ from them, however, in our interpretation of the first and more striking change which has apparently not previously been recognized as a different phenomenon.

Our reasons for considering the rapid initial change as due to an ionic exchange rather than to a leakage of salts are: first, its rapidity and definiteness; second, the fact that it occurs in isosmotic as well as in hypotonic sucrose solutions, whereas, according to Ponder and Saslow (1931), leakage of salts in general takes place only in the latter, in proportion to their degree of hypotonicity; and third, and most important, the fact that this effect is readily reversible while the second and slower one is not. A typical experiment showing this reversibility is summarized in Table V. As indicated in this table, an exposure of ox erythrocytes for 3 minutes to 0.2 M sucrose reduced the degree of hemolysis in 0.125 M sucrose from 61 per cent to 20 per cent. For a time this new level was maintained before falling further. If, however, the cells after a 3 minutes exposure to 0.2 M sucrose were placed in 0.2 M sucrose + 0.002 M NaCl for varying times and the resulting suspension was then suddenly diluted to 0.125 M sucrose + 0.001 M NaCl, the percentage of hemolysis returned to about its initial

TABLE V

Ox erythrocytes exposed to 0.2 M sucrose for 3 minutes and then for varying additional times to 0.2 M sucrose containing 0.002 M NaCl. Final hemolysis in 0.125 M sucrose or in same + 0.001 M NaCl. Temperature, 20° C.

Time of exposure	Final percentage of hemolysis
No initial exposure to 0.2 M sucrose	61
3 minutes exposure to 0.2 M sucrose	20
Same after the following further exposures to 0.2 M sucrose + 0.002 M NaCl	
30 seconds	53.5
1 minute	60
2 minutes	60.5
5 minutes	55.0
15 minutes	54.0
30 minutes	52.0
40 minutes	52.5
50 minutes	47.5
100 minutes	44.5
160 minutes	34.5
255 minutes	12.0

value. The same general results were obtained when the final solution was 0.125 M sucrose + 0.002 M NaCl. In other experiments it was found that $CaCl_2$ is roughly twice as effective as NaCl in reversing the non-electrolyte effect and that times of considerably less than 30 seconds are sufficient to produce the change.

If it be supposed that the observed rise in the osmotic resistance of the erythrocytes during a 3-minute exposure to 0.2 M sucrose is due to a leakage of salts from cells that have lost their normal impermeability to cations, then it is extremely difficult to see how the original properties of the cells can so easily be restored by the simple treatment described above. The explanation of an ionic exchange between essentially normal cells and their surroundings seems much more plausible. It is to be noted that the later and slower increase in resistance, which we have interpreted as being due to an escape of salts, cannot be reversed in this way. This is shown not only by experiments in which the original exposure to 0.2 M sucrose is of long duration, *e.g.*, an hour, but also by the fairly rapid falling off of the figures in Table V. Evidently, even in the presence of added NaCl, the high degree of hemolysis at first produced cannot be maintained, which is what would be expected if salts slowly escape from the cells into the solution.

V

It remains to consider the manner in which a mere reversible exchange of ions between an unaltered erythrocyte and its surroundings could simulate the osmotic effect produced by an irreversible escape of salts or other osmotically active materials. One possible method by which this effect might be produced in a cell permeable to anions and not to cations was suggested in principle many years ago by Hamburger (1902) and is further discussed by Ege (1921). If, for example, SO_4'' ions from outside the cell were exchanged for electrically equivalent quantities of Cl' and HCO_3' ions from inside the cell, the internal osmotic pressure would be lowered, since the exchange would be in a 1 : 2 ratio. Though this method illustrates the possibility of a decrease in internal osmotic pressure without "leakage" and without any other change in the normal properties of the cell, it obviously cannot apply to non-electrolyte solutions in which the only ions present are $H^{\cdot}$ and OH', both of which are univalent. However, though the exchange of Cl' or HCO_3' from the cell for OH' from the solution can produce no direct osmotic effect (see Jacobs and Parpart, 1932, for evidence that $H^{\cdot}$, as such, does not enter the cell), it by no means follows that an indirect effect is impossible. It will now be shown that, as a matter of fact, such an indirect effect is to be expected.

(In this connection see also Jacobs, 1932, and, for an important contribution to the general theory, Netter, 1928.)

It is almost universally admitted (for a summary of the evidence see Jacobs, 1931) that, whatever may be true of the erythrocyte in abnormal surroundings, in the body it is freely permeable to at least most anions and practically impermeable to cations. It is also generally agreed that of the total base contained within the cell, a part under usual conditions is combined with hemoglobin to form a dissociated salt-like compound, and most of the remainder is paired with inorganic anions such as Cl' and HCO_3'. The proportions of the total base bound by hemoglobin, on the one hand, and by other acids, on the other, are subject to rather wide variations with changes in pH, temperature, oxygenation of the hemoglobin, etc.; these variations are of importance in connection with the normal physiological transport of CO_2 by the blood. When we add the further well-established fact that the osmotic pressure of a solution of the salt of hemoglobin, which may be represented as BnHb, is much less than that of a solution containing the same amount of base in the form of a uni-univalent salt, *e.g.*, of n BCl or n $BHCO_3$, we have all the facts necessary for a plausible theory as to the apparently peculiar behavior of the erythrocyte in non-electrolyte solutions.

To avoid unnecessary complexity we may consider the system:

$$\mathrm{HHb} + \mathrm{BHb} + \mathrm{BCl} \mid \text{non-electrolyte}$$

in which all the inorganic salts of the erythrocyte are for simplicity treated as chloride. The system is otherwise supposed to have the general properties just mentioned. By hypothesis, the membrane which separates the two solutions is permeable to the anions Cl' and OH' but not to cations nor to hemoglobin in any form. Under these conditions, by the principle of Donnan, the equilibrium

$$\frac{[\mathrm{Cl}]\ \text{inside}}{[\mathrm{Cl}]\ \text{outside}} = \frac{[\mathrm{OH}]\ \text{inside}}{[\mathrm{OH}]\ \text{outside}} = \frac{[\mathrm{H}]\ \text{outside}}{[\mathrm{H}]\ \text{inside}}$$

will tend to be established. Following the usual approximate treatment of such problems, we shall consider the quantities within the square brackets to represent concentrations rather than activities, from which they do not here differ very greatly numerically.

In the attainment of the equilibrium condition there must be an exchange of OH' from the external solution for Cl' from the cell. But such an exchange leads to the reaction:

$$\begin{array}{r|ll|l}
\mathrm{HHb} & & \rightarrow \mathrm{K^{\cdot}Hb'} & \\
\mathrm{K^{\cdot}} & \mathrm{H^{\cdot}} & \mathrm{H_2O} & \mathrm{H^{\cdot}} \\
\mathrm{Cl'} & \mathrm{OH'} \leftarrow & & \mathrm{Cl'}
\end{array}$$

which evidently diminishes the internal osmotic pressure. No salt has left the cell and the ions Cl′ and OH′ have been exchanged in amounts which are exactly equivalent osmotically, and yet the somewhat paradoxical result appears that the cell has undergone an osmotic change in the same direction as if it had suffered a leakage of salts.

VI

It would seem that there could be little theoretical objection to the mechanism just suggested, which is deduced entirely from generally accepted properties of the erythrocyte. The only question likely to be raised is as to whether the magnitude of the effect so produced could be great enough to account for the observed initial behavior of the erythrocyte in non-electrolyte solutions. This question may be at least partly answered by a simple semi-quantitative treatment of the equilibrium in question. It is unfortunately not yet possible to make calculations of completely quantitative exactness; this difficulty is due both to the complexity of the erythrocyte and to the insufficiency of the existing data. Nevertheless, at least the general principles governing all such systems will emerge from the calculations about to be made.

For simplicity let us start with what initially amounts practically to a unit volume of cell contents, exclusive of the "osmotic dead space" referred to above, *i.e.*, with a quantity of cells containing a unit amount of water. The volume of the cell water, which may be represented by V_c, will vary during the course of the experiment with changes in osmotic pressure within the cells. The amount of the external non-electrolyte solution is assumed to be so large that its volume, V, will undergo no appreciable changes. This condition is actually realized in the experiments described above in which the cells were suspended in over 1000 times their own volume of solution. The external solution at the beginning of the experiment will be assumed to be exactly neutral and isosmotic with the blood. Cases where it is not initially isosmotic can be treated by imagining that before any other changes occur the cells gain or lose enough water to bring them into temporary osmotic balance with the surrounding solution; this treatment is justified because we are dealing with a final equilibrium and the position of such an equilibrium is not affected by the order in which the various supposedly reversible changes that lead to it occur.

At equilibrium we must have:

$$\frac{[\mathrm{Cl}]\ \text{inside}}{[\mathrm{Cl}]\ \text{outside}} = \frac{[\mathrm{H}]\ \text{outside}}{[\mathrm{H}]\ \text{inside}}. \tag{1}$$

For convenience $H^{\cdot}$ ions rather than OH' ions have been used in this equation because, although the cell is believed to be impermeable to them, their concentration at all times bears the fixed relation to that of OH' represented by $[H][OH] = K_w$. There may now be substituted for the four quantities in brackets in equation 1 expressions involving V_c and V, already defined, and the amounts of hemoglobin, a, total base, b, base bound by hemoglobin, b', and chlorine, c, initially present in the cell, together with the amount, x, of Cl' exchanged during the course of the experiment for OH'. The resulting equation may then be solved for x and the magnitude of the resulting osmotic effect estimated.

The equilibrium value of $[Cl]$ inside is obviously $(c - x)/V_c$. But the volume of liquid in the cell is supposed to be determined according to ordinary osmotic laws by the quantities of solutes present. If the initial volume is unity, V_c must therefore be equal to $(a + b + c - x)/(a + b + c)$. It will be noted that a and b undergo no change, since the cell is impermeable to hemoglobin in all forms and to cations; x therefore represents the total change in the solutes in the cell. The value of $[Cl]_{\text{outside}}$ is the simplest of the four quantities in brackets, being merely x/V. Of the quantities on the right-hand side of the equation, $[H]_{\text{outside}} = [Cl]_{\text{outside}} + [OH]_{\text{outside}}$, or, substituting x/V for $[Cl]$ and $K_w/[H]$ for $[OH]$ we have:

$$[H]_{\text{outside}} = \frac{\sqrt{K_w + \frac{x^2}{4}} + \frac{x}{2}}{V}.$$

For practical purposes, since K_w in our problem is very small compared with $x^2/4$, no appreciable error is caused by placing $[H]_{\text{outside}} = x/V$. This is the same as saying that $[H]_{\text{outside}}$ is practically the same as $[Cl]_{\text{outside}}$.

We have now only to find the value of $[H]$ inside in terms of the quantities already used in order to have a complete equation from which x may be determined. Fortunately, the reaction $HHb + K^{\cdot} + OH' = K^{\cdot} + Hb' + H_2O$ is practically complete within the pH range dealt with here, so x, the amount of OH' that enters the cell, is with no appreciable error, equal to the additional base combined with hemoglobin over and above the amount, b', initially present. For the relation between pH and the amount of base combined with hemoglobin we have selected as sufficiently accurate for our purposes, the simple equation given by Van Slyke, Hastings, Heidelberger, and Neill (1922) in place of their more complicated one published later.

The equation used is that for oxyhemoglobin, since oxygenation is almost complete under the conditions of our experiments. It is:

$$\text{Total base combined} = b' + x = 2.6 \text{ Hb } (\text{pH} - 6.6). \qquad (2)$$

This may be rewritten

$$\text{pH} = \frac{b' + x}{2.6 \text{ Hb}} + 6.6$$

and finally,

$$[\text{H}]_{\text{inside}} = 10^{-\left(\frac{b'+x}{2.6\,\text{Hb}}+6.6\right)}.$$

Conditions within the erythrocyte are doubtless somewhat different from those for which this equation was intended to apply, but probably not sufficiently so to deprive our calculation of at least a semi-quantitative significance. Though the equation describes the behavior of hemoglobin at 38° C. and our experiments were made at 20° C., this difference is not as serious as it might appear to be, since we are interested primarily in *changes* in base bound by hemoglobin and, according to Stadie and Martin (1924), the changes in BHb with change in pH, *i.e.*, the buffer value of hemoglobin, are scarcely affected by moderate temperature changes.

Combining now the values separately found for the four quantities in brackets in equation (1) we have, after transferring $[\text{H}]_{\text{outside}}$ to the left-hand side:

$$\frac{x^2(a + b + c - x)}{V^2(a + b + c)(c - x)} = 10^{-\left(\frac{b'+x}{2.6\,\text{Hb}}+6.6\right)}. \qquad (3)$$

By the substitutions of appropriate numerical values for a, b, c, V and b', equation 3 may be solved for x by any of the usual methods for the solution of transcendental equations (see in this connection Scarborough, 1930). The values here chosen have mostly been taken in round numbers from the data given by Henderson (1928, pages 195–6) after reducing them to amounts per unit volume of cell water. For b we have taken 0.160 and for c, 0.080 mols per liter of water. For total hemoglobin in the equation used for the calculation of combined base, we have taken 0.030, which is approximately Henderson's figure, and is measured in terms of the combining power of hemoglobin for oxygen; for a, on the other hand, which is a measure of the osmotic effectiveness of the total hemoglobin, a lower figure is necessary and we have somewhat arbitrarily chosen one of 0.010; the exact value assigned to a is, as a matter of fact, within reasonable limits, of little consequence. The calculated amount of base combined with hemoglobin, b', at pH 7.1, the assumed initial intracellular pH, proves to be somewhat less than Henderson's figure, but to be consistent we have used equation 2 throughout. The value so calculated is 0.039.[1]

In Table VI are given the calculated equilibrium values of x and V_c and of the concentrations of Cl′ and H′ inside and outside the hypothetical simplified erythrocytes for two selected values of V. The larger one represents approximately the conditions of the experiments where one drop of blood was suspended in 25 cc. of solution. It is obvious that under these conditions the osmotic effect of the ionic exchange in question is very considerable. The calculated volume change in Table VI for $V = 1000$ is approximately 15 per cent. This change would be more than enough to account for the rapid initial decrease in the percentage of hemolysis shown in Table IV, since in the preliminary standardization of the blood used in this experiment it was found that an increase in concentration from 0.125 M to 0.135 M sucrose (*i.e.* about 8 per cent) reduced the percentage of hemolysis by about the same amount as the 3-minute pre-hemolytic exposure to 0.2 M sucrose.

TABLE VI

Equilibrium values calculated by means of equation 3 for the two cases: $V = 100$ and $V = 1000$.

	$V = 100$	$V = 1000$
x	0.00697	0.0368
V_c	0.972	0.853
$[Cl]_0$	6.97×10^{-5}	3.68×10^{-5}
$[Cl]_i$	7.51×10^{-2}	5.07×10^{-2}
$[Cl]_i/[Cl]_0$	1.08×10^{3}	1.37×10^{3}
$[H]_i$	6.47×10^{-8}	2.68×10^{-8}
$[H]_0$	6.97×10^{-5}	3.68×10^{-5}
$[H]_0/[H]_i$	1.08×10^{3}	1.37×10^{3}

It should be strongly emphasized that no importance is attached by us to anything but the order of magnitude of the calculated values of x and V_c. In the first place, the hypothetical system for which the calculations have been made differs from the erythrocyte in several important respects; for example, the CO_2-bicarbonate system has purposely been omitted from it. Furthermore, it is impossible under actual experimental conditions to expose erythrocytes to pure non-electrolyte solutions—at least without first altering them by excessive washing, since otherwise some salts will of necessity accompany them—and Table VI as well as the experiment on which Table V is based show the important effect of very low concentrations of electrolytes in the external solution. Finally, there is the difficulty, discussed in an earlier paper (Jacobs, 1932) that even during the process of hemolysis in a non-electrolyte solution a change in osmotic resistance may occur; and for this reason the degrees of hemolysis observed in the controls may be affected in a way that is far from simple. It would seem impracticable, therefore, at least by the present type of experiments, to make a thoroughly satisfactory quantitative test of the correctness of the theory here advanced. We believe, however, that the calculations that have been made, rough as they admittedly are, will at least serve to indicate that a reversible ionic exchange is not unworthy of further consideration as one of the factors responsible for the apparently anomalous osmotic behavior of the erythrocyte.

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[1] This value of b' gives rise to the difficulty that the sum of b' and c is not equal to b as it should be. However, even if this equality had been preserved by reducing b to 0.119, the effect on Table VI would have been so slight as to be scarcely noticeable; the new values of x in this case would have been 0.00699 and 0.0372 and those of V_c 0.967 and 0.822 instead of the ones given. From the manner in which b enters into equation 3, its exact value over a considerable range, like that of a, is relatively unimportant.

We are very glad to acknowledge the valuable assistance of Mr. Samuel A. Corson in connection with many of our experiments.

Summary

1. The degree of hemolysis of ox erythrocytes produced by a given hypotonic NaCl solution is not decreased by a previous exposure of from a few seconds to several hours to a similar but slightly less hypotonic non-hemolytic solution. Within this period the hemolysis method gives no evidence of any escape of osmotically active substances from the cells. After 3 or 4 hours in the case of ox erythrocytes, and from a few minutes to an hour in the case of those of the pig, cat, rabbit and man, there may be a gradual increase in osmotic resistance suggestive of a slow escape of salts.

2. Solutions of sucrose, either hypotonic or isotonic, cause a striking increase in the osmotic resistance of erythrocytes; this occurs in two stages: a rapid one and a much slower one which follows the first after a considerable interval. While the slower change is probably associated with an escape of salts, the more rapid and more striking one, which is readily reversible, is interpreted as being due to an exchange of anions from the cell for OH′ ions from the solution, the

resulting increase in base bound by hemoglobin causing a decreased osmotic pressure within the cell. Calculations show that the expected osmotic effect of such an exchange is of the order of magnitude of that actually observed.

LITERATURE CITED

BANG, I., 1909. *Biochem. Zeitschr.*, **16**: 255.

CSAPÓ, J., AND E. KERPEL-FRONIUS, 1933. Beiträge zur Physiologie des Saüre-Basenhaushaltes und der Osmoregulation. *Pflüger's Arch.*, **231**: 662.

EGE, R., 1921. Untersuchungen über das Volumen der Blutkörperchen in gegenseitig osmotischen Lösungen. *Biochem. Zeitschr.*, **115**: 109.

EGE, R., 1922. Untersuchungen über die Volumen veränderungen der Blutkörperchen im Lösungen von verschiedenen osmotischen Druck. *Biochem. Zeitschr.*, **130**: 99.

GOUGH, A., 1924. The Nature of the Red Blood Corpuscle. *Biochem. Jour.*, **18**: 202.

GREEN, R. G., 1925. *Proc. Soc. Exper. Biol. Med.*, **22**: 308.

HAMBURGER, H. J., 1902. Osmotischer Druck und Ionenlehre. Vol. L, pp. 161–400. Wiesbaden.

HENDERSON, L. J., 1928. Blood: a Study in General Physiology. New Haven.

HILL, A. V., 1930. The State of Water in Muscle and Blood and the Osmotic Behaviour of Muscle. *Proc. Roy. Soc.* B, **106**: 477.

JACOBS, M. H., 1930. Osmotic Properties of the Erythrocyte. I. Introduction. A simple method for studying the rate of hemolysis. *Biol. Bull.*, **58**: 104.

JACOBS, M. H., 1931. The Permeability of the Erythrocyte. *Ergebn. d. Biol.*, **7**: 1.

JACOBS, M. H., 1932. Osmotic Properties of the Erythrocyte. III. The applicability of osmotic laws to the rate of hemolysis in hypotonic solutions of non-electrolytes. *Biol. Bull.*, **62**: 178.

JACOBS, M. H., AND A. K. PARPART, 1931. Osmotic Properties of the Erythrocyte. II. The influence of pH, temperature, and oxygen tension on hemolysis by hypotonic solutions. *Biol. Bull.*, **60**: 95.

JACOBS, M. H., AND A. K. PARPART, 1932. Is the Erythrocyte Permeable to Hydrogen Ions? *Biol. Bull.*, **62**: 63.

JOEL, A., 1915. Über die Einwirkung einiger indifferenter Narkotika auf die Permeabilität roter Blutkörperchen. *Pflüger's Arch.*, **161**: 5.

KERR, S. E., 1929. Studies on the Inorganic Composition of Blood. III. *Jour. Biol. Chem.*, **85**: 47.

KREVISKY, C., 1930. The Determination of the Quantity of Free Water in Erythrocytes. *Biochem. Jour.*, **24**: 815.

MACLEOD, J., AND E. PONDER, 1933. The Measurement of Red Cell Volume. IV. *Jour. Physiol.*, **77**: 181.

NETTER, H., 1928. Über die Elektrolytgleichgewichte an elektiv ionenpermeabelen Membranen und ihre biologische Bedeutung. *Pflüger's Arch.*, **220**: 107.

PONDER, E., AND G. SASLOW, 1930. The Measurement of Red Cell Volume. II. *Jour. Physiol.*, **70**: 169.

PONDER, E., AND G. SASLOW, 1931. The Measurement of Red Cell Volume. III. *Jour. Physiol.*, **73**: 267.

SCARBOROUGH, J. B., 1930. Numerical Mathematical Analysis. Baltimore.

STADIE, W. C., AND K. A. MARTIN, 1924. The Thermodynamic Relations of the Oxygen-and-Base-combining Properties of Blood. *Jour. Biol. Chem.*, **60**: 191.

VAN SLYKE, D. D., A. B. HASTINGS, M. HEIDELBERGER, AND J. M. NEILL, 1922. Studies of Gas and Electrolyte Equilibria in the Blood. III. *Jour. Biol. Chem.*, **54**: 481.

NOTES ON THE CENTRIOLES OF AMPHIBIAN TISSUE CELLS

ARTHUR W. POLLISTER

Department of Zoölogy, Columbia University

Introduction

The central apparatus was a favorite object of cytological study for about thirty years following its discovery by Van Beneden in 1876. The extensive literature on this subject during that period included a considerable body of data tending to the conclusion that a part of the central apparatus, the pair of centrioles, is a constant component of most metazoan cells. These data, chronologically tabulated by Heidenhain in 1907, form an impressive array including examples of centrioles in many invertebrates and in practically every type of vertebrate somatic tissue cell. I have recently been able to confirm many of these results in studies on the favorite object for histological researches, the tissues of Amphibia; and in view of the fact that the earlier results have received practically no detailed attention in American cytological literature it seemed to me that it might be worth while to describe my observations briefly.

The greater part of the literature on centrioles has been concerned entirely with the behavior of these bodies during mitotic cell division. Curiously enough, researches demonstrating that the centrioles found in non-dividing cells actually separate and take positions at the ends of the mitotic spindle are rather rare. Accordingly I have included in Part II a description of a very clear case of this sort.

Material and Methods

The centrioles of non-dividing cells have all been drawn from serial sections of a 14 mm. larva of *Ambystoma opacum*. By this stage the animal has been feeding for some time, yolk resorption is completed, and most of the tissues are essentially like those of the adult. The animal was fixed in Benda's fluid, mordanted, and stained by the alizarin-crystal violet method. The slides were differentiated especially for demonstration of centrioles, which are stained an intense violet by this method. In such a preparation only the following cytoplasmic structures are stained in addition to the centrioles: myofibrillæ of striated muscle fibres, prozymogen granules of the pancreas, granules in the cells of the first part of the proximal segment of the pronephric and mesonephric tubules, and the eosinophilic granules of leucocytes. Accordingly, in most cells the cytoplasm is almost completely colorless and homogeneous except for the two deep purple centrioles. Occasionally the chondriosomes are visible though unstained. But in most cells, although these bodies are well preserved, as can be demonstrated by heavier staining with the crystal violet, the chondriosomes are indistinguishable when unstained, presumably because their refractive index is almost exactly that of the general cytoplasm. I have made all the drawings from this one animal for the very obvious reason that such a method is of itself almost a completely adequate refutation of any doubt as to the homology of the centrioles in the different tissue cells. Indeed on any one section there are always examples of centrioles in a considerable number of different cells.

The study of the leucocyte divisions (Part II) has been made from the leucopoietic capsule of the liver of *Amphiuma tridactylum* also prepared by the Benda method.

OBSERVATIONS

I. Centrioles of Non-dividing Cells

Connective Tissues.—The fibroblast (Fig. 1) contains a pair of centrioles in a small volume of cytoplasm closely adjacent to the nucleus. This agrees with the observations of Spuler (1896) on the mammalian fibroblast. The cartilage cell (Fig. 2) contains a pair of large centrioles either near the surface of the cell as here figured or in an internal position near the nucleus. Wherever located the centrioles are surrounded by an area of cytoplasm that stains more heavily than the exceptionally clear remainder of the cell. This region is obviously the same as the more sharply demarcated sphere of cytoplasm described by von Smirnow (1906) in the cartilage cells of *Proteus.*

Smooth Muscle.—This cell, of which only a part is shown in Fig. 3, contains a pair of small centrioles located opposite a point approxi-

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EXPLANATION OF PLATES I–VI

The figures are all camera lucida drawings made at the table level with a Zeiss 120 objective and a 20× ocular. This gives a magnification of approximately 3600 diameters. The figures have been reduced one-half in reproduction. The epithelial cells of Plates II and III have all been mounted so that the lumen end of the cell faces the top of the plate. The figures of Plates I, II, and III have been drawn from serial sections of a 14 mm. larva of *Ambystoma opacum* prepared by the Benda method. Plates IV, V, and VI were drawn from slides of the liver of *Amphiuma tridactylum* prepared by the same method.

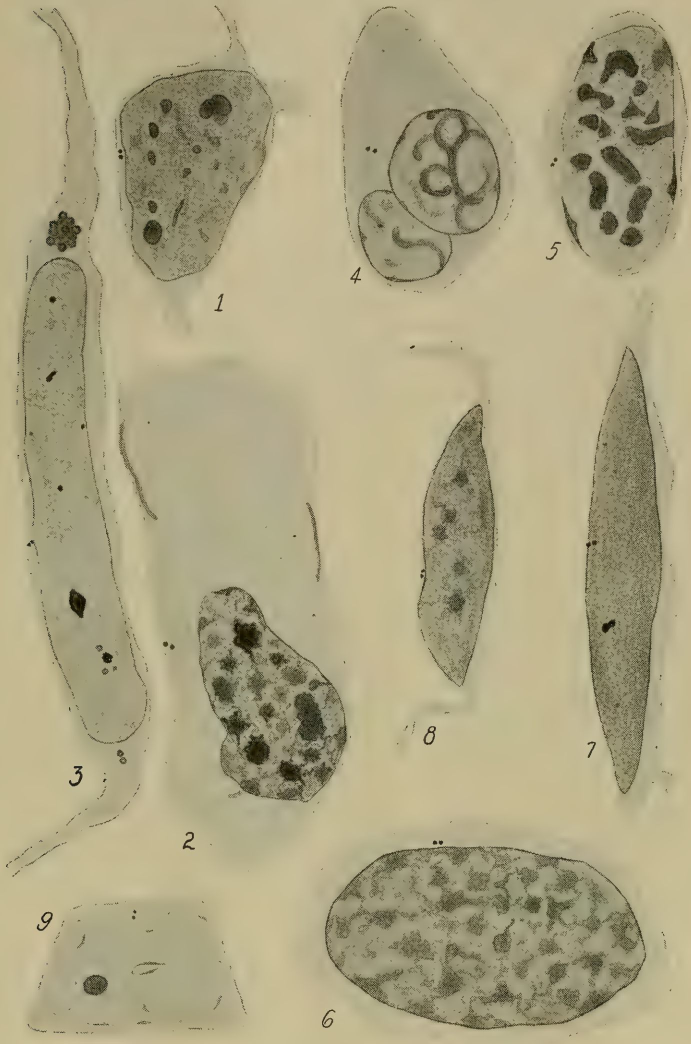


Plate I

Explanation of Figures

1. Fibroblast from the submucosa of the wall of the stomach.
2. Cartilage cell from the basisphenoid cartilage.
3. Smooth muscle fibre from the wall of the stomach.
4. Non-granular leucocyte from the capsule of the liver.
5. Thrombocyte from the atrium.
6. Epithelial cell from the serosa of the stomach.
7. Epithelial cell from the lining of the pericardium.
8. Endothelial cell from branchial vessel.
9. Cell from the lower layer of the stratified squamous epithelium lining the pharynx.

mately midway along the length of the nucleus, where there is frequently a small depression in the nucleus. This observation agrees exactly with the results of von Lenhossék (1899).

Blood Cells.—The non-granular leucocyte of *Ambystoma opacum* (Fig. 4) has a nucleus that is roughly kidney-shaped and located in one side of the cell. The two centrioles lie nearly in the center of the cytoplasm opposite the concave surface of the nucleus. By study of other preparations it becomes clear that the centrioles are enclosed in a sphere as in *Amphiuma* (Fig. 26). The leucocyte centrioles have been more extensively studied than those of any other vertebrate tissue. Heidenhain (1907), who has given them most detailed attention, frequently found cells that apparently contained three centrioles. I have never seen any cell that had more than two and I think that Heidenhain's results can be completely understood when one realizes that he used the iron hematoxylin method of staining centrioles. As shown in Fig. 26, closely adjacent to the centrioles there is a third body, which I have somewhat inaccurately called a centrosome (Pollister, 1932*a*), and which is the focal point of the rays of the large aster which is characteristic of amphibian leucocytes. This centrosome often stains as heavily with iron hematoxylin as do the two centrioles and in my slides prepared by this method one sees a majority of the cells with three granules in the position of the centrioles. With the Benda method, however, it is easily seen that one of these is the centrosome and two are the centrioles since only the latter are stained by the crystal violet.

The thrombocyte (Fig. 5) of *Ambystoma opacum* contains a pair of small centrioles opposite the middle of the nucleus. I have been unable to identify the centrioles in the erythrocyte owing to the fact that the entire cytoplasm of this cell stains very heavily with the crystal violet.

Epithelial Tissues.—The peritoneal epithelial cell (Fig. 6) usually contains a pair of centrioles not far to one side of the nucleus, exactly as figured by Flemming (1891) in the first observations of centrioles in somatic cells. In some instances (Fig. 7) these bodies are located between the nucleus and the free surface of the cell adjacent to the cœlomic cavity. Endothelial cells (Fig. 8) have a pair of centrioles located between the nucleus and the lumen of the blood vessel.

The surface cells of the stratified squamous epithelium lining the pharynx of *Ambystoma opacum* (Fig. 10) contain a pair of centrioles surrounded by a clear sphere and located just within the lower part of the cuticle at the free surface of the cell. The deeper cells (Fig. 9) likewise have the centrioles situated between the nucleus and the

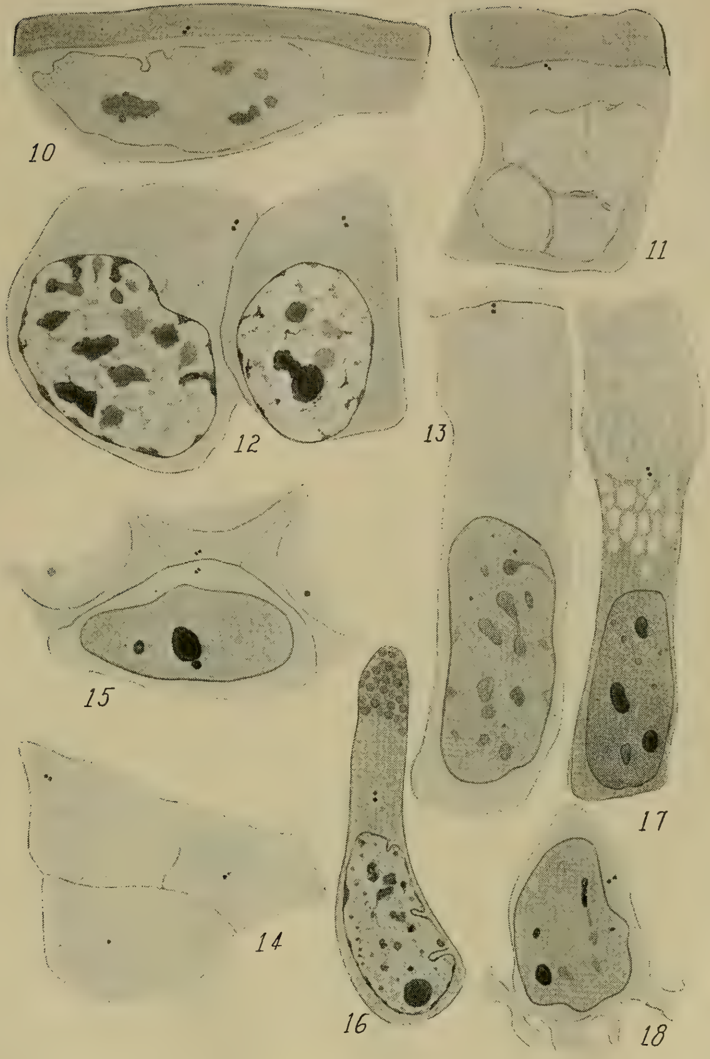


PLATE II

Explanation of Figures

10. A surface cell of the stratified squamous epithelium lining the pharynx.
11. Cell from the surface epithelium of the stomach.
12. Two cells of a gastric gland.
13. A cell from the surface epithelium lining the intestine.
14. Three cells of the surface epithelium of the small intestine seen on end from the direction of the lumen of the intestine.
15. Section of an intestinal gland showing all of one cell and parts of three others.
16. Mucous cell from the œsophagus.
17. Goblet-cell from the small intestine.
18. Epithelial cell from the lining of the unexpanded larval lung.

direction of the free surface of the epithelial layer. Both these observations agree exactly with those of Zimmerman (1898) and Joseph (1903).

The surface epithelial cells of the stomach (Fig. 11) have the end adjacent to the lumen specialized as a thick cuticle. Immediately under this cuticle is a pair of centrioles so arranged that one is always nearer the nucleus than the other. There are two types of cells in the gastric glands of *Ambystoma opacum*. These two are drawn in Fig. 12. Each cell contains a pair of centrioles between the nucleus and the lumen end of the cell, somewhat nearer the latter.

The surface epithelial cells of the intestine (Fig. 13) have a specialized border on the free surface and a pair of centrioles in exactly the same topographical relationship as is found in the gastric mucosa. This situation agrees with the descriptions given by Zimmerman (1898), and by Heidenhain (1907). As shown in Fig. 14 the centrioles are located approximately in the center of the tip of the cell in most cases. The intestinal gland cells (Fig. 15) contain a pair of centrioles surrounded by a small sphere of more lightly-staining cytoplasm and located in the same region of the cell as the centrioles of the gastric glands.

In mucous cells the centrioles are always situated deep within the cytoplasm, some distance from the lumen end of the cell—in marked contrast to the other cells in direct contact with the digestive cavity. They are generally near the proximal end of the mass of mucus that occupies the tip of the cell as previously described by Zimmerman (1898), Lenhossék (1898), Joseph (1903), and Tschassownikow (1914). A mucous cell from the oesophagus is shown in Fig. 16 and the typical goblet-cell of the intestine is illustrated in Fig. 17. I have been unable to confirm Joseph's description of a fibre running toward the lumen end of the cell from the distal centriole and another fibre extending toward the nucleus from the proximal centriole. It is interesting to note that Heiderich (1910) has seen these goblet-cell centrioles in freshly teased, unfixed preparations of frog tissues.

The cell of the peculiar epithelium of the unexpanded lung of the larval *Ambystoma opacum* (Fig. 18) has a pair of centrioles between the nucleus and the lumen, as in the other endodermal cells that have been described above.

Flagellated Epithelial Tissues.—In many epithelial cells the distal centriole serves as a blepharoplast to which a flagellum is attached. Figures 20 and 21 show two such cells from the pancreatic duct. These should be compared with Fig. 19 from the same region. The resemblance between the centrioles of these flagellated and non-flagel-

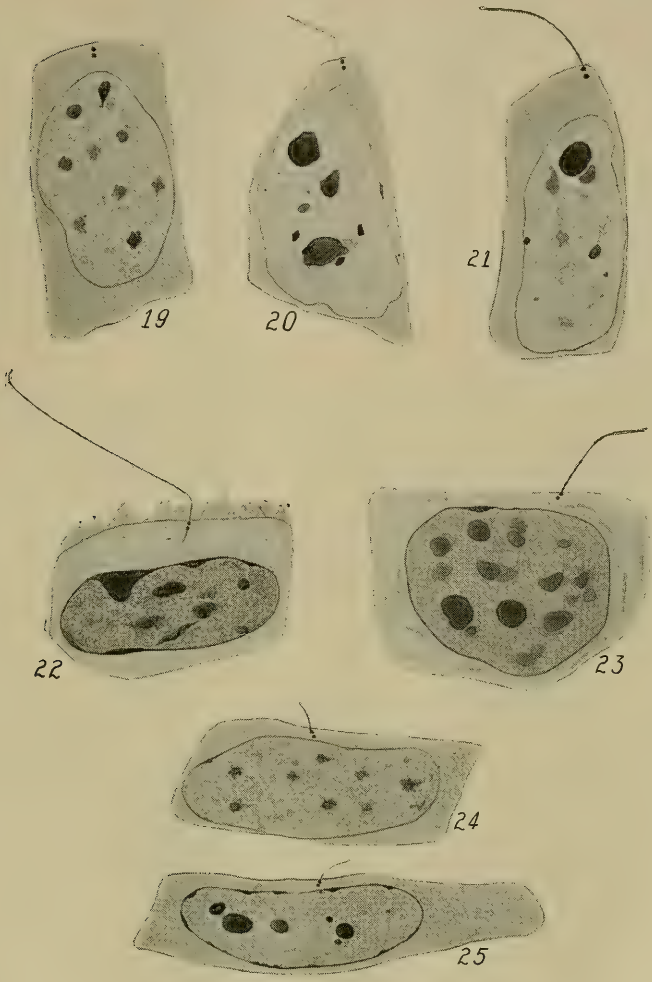


Plate III

Explanation of Figures

19. Non-flagellated cell from the pancreatic duct.
20, 21. Flagellated cells from the pancreatic duct.
22. Flagellated cell from the proximal segment of the pronephric tubule.
23. Flagellated cell from segmental duct.
24. Flagellated cell from the distal segment of the pronephric tubule.
25. Flagellated cell from the lining of the otic vesicle.

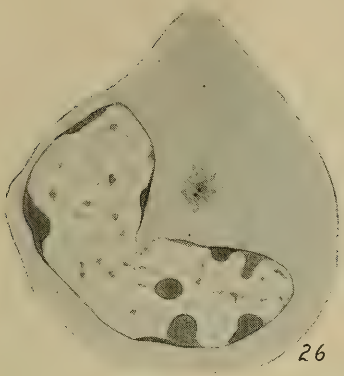
lated types is very convincing evidence that they are strictly homologous structures, differing only in the existence of a flagellum attached to the distal centriole in one type. Nearly every cell in the larval pronephros and mesonephros is flagellated (Figs. 22, 23, and 24). Because this study has been made from sectioned material it has been impossible to determine the characteristic length of the flagellum. The longest I have observed was more than twice the length of that in Fig. 22. This cell is of further interest as being the only instance where I have been able to find a fibre running inward toward the nucleus from the proximal centriole as described by Joseph (1903) in the ureter of *Torpedo*. In Fig. 25 is shown an example of a flagellated cell from the lining of the otic vesicle.

II. *The Behavior of Centrioles during Division of the Non-granular Leucocytes of Amphiuma tridactylum*

The production of both granular and non-granular leucocytes in the adult *Amphiuma* takes place largely in a layer of tissue, several cells in thickness, lying immediately below the peritoneal investment of the liver. As in any leucopoietic center, mitotic figures are frequent in this locality. The behavior of the centrioles during the mitosis of these cells has already been somewhat schematically figured and briefly described by Bělăr (1926) in *Salamandra;* and the writer has described the present case in a short note without figures (Pollister, 1932*a*).

The centrioles of the non-dividing leucocyte (Fig. 26) are near the center of a roughly spherical mass of more heavily-staining cytoplasm, the centrosphere. In cells with kidney-shaped nuclei the centrioles-centrosome-centrosphere complex is located opposite the concavity of the nucleus as described earlier for *Ambystoma opacum* (see p. 532). The nucleus in many instances, however, is in the form of a complete ring, and in such a case the sphere lies either just outside or actually within the hole in the ring. In either case it seems likely that Heidenhain's contention that the centriole always lies approximately in the center of the cell expresses the situation quite clearly. These special leucocytes of *Amphiuma* are probably the largest found in any vertebrate. It is not infrequent to find a single cell running through as many as five sections, each six microns in thickness. In the plates, accordingly, no one cell is completely figured although in every case I have identified and studied the remainder of the cell in the adjacent sections.

The condensation of chromatin prior to actual chromosome formation has proceeded to some extent before there is any change of position of the centrioles (Fig. 27). During the later prophase stages the centrioles move apart to positions at opposite sides of the nearest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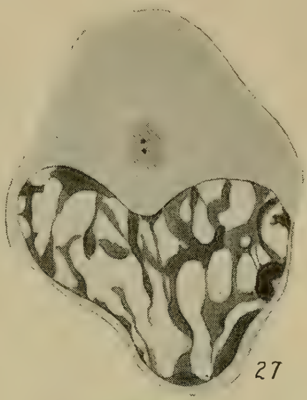
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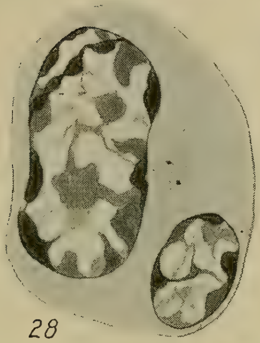
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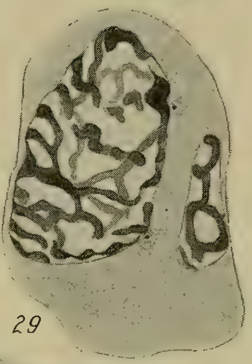
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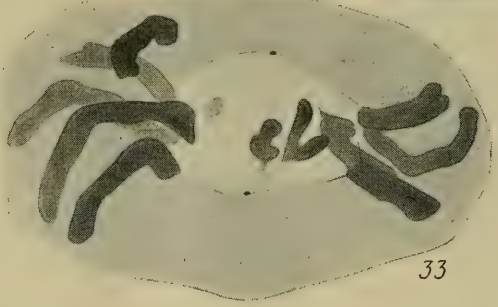
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PLATE IV

Explanation of Figures

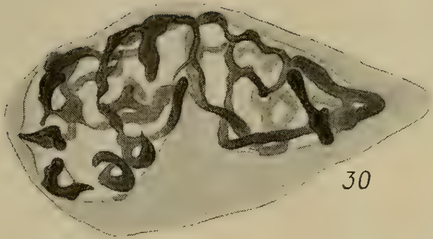
26. Non-dividing leucocyte showing sphere, centrosome, and two centrioles.

27. Early prophase. The sphere and centrosome have disappeared. Centriole migration has not yet begun.

28. Later prophase. Early stage in separation of the two centrioles.

29. Later prophase. Intermediate stage in centriole migration.

33. Metaphase stage.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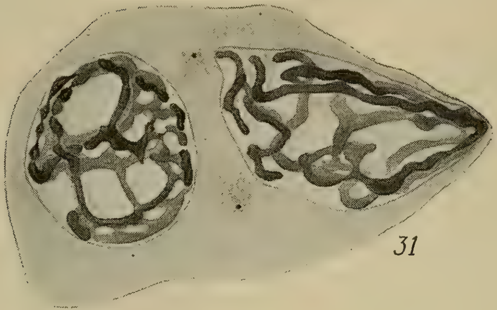
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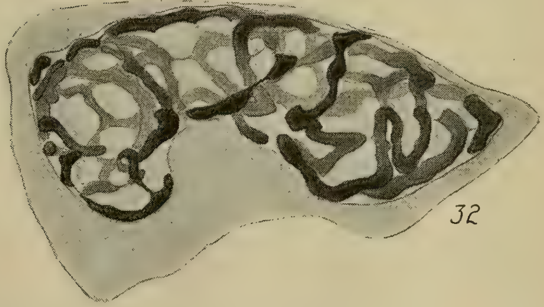
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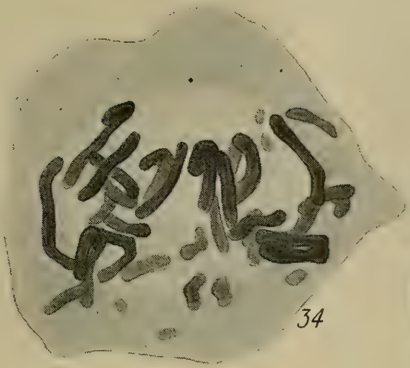
Plate V

Explanation of Figures

30, 31, and 32. Three successive sections of a late prophase of a leucocyte with a ring-shaped nucleus. Beginning of development of the central spindle between the two centrioles.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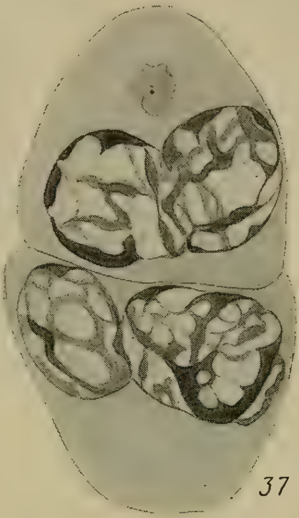
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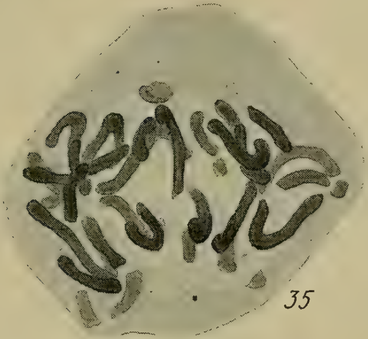
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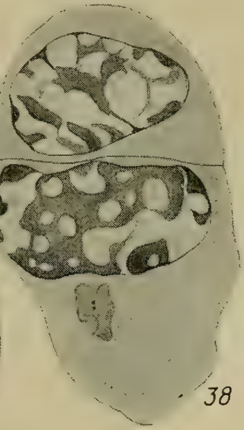
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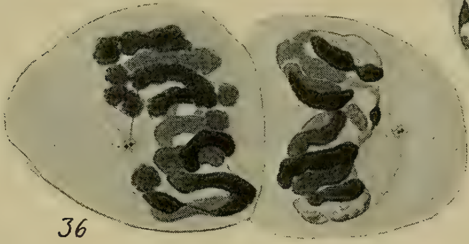
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PLATE VI

Explanation of Figures

34, 35. Two sections of a cell in the anaphase stage.

36. Two sister cells in early telophase. Centrioles doubled in each cell.

37, 38. Two successive sections through two sister cells in late telophase. Sphere reconstituted about the centrioles in each cell.

adjacent part of the nucleus (Figs. 28 and 29). In the case of a cell with a ring nucleus the centrioles migrate in opposite directions through the hole in the ring, and when this movement is completed the centrioles are no longer actually within the ring, but are located just outside opposite ends of the hole through the ring (Figs. 30, 31, and 32). At this stage, which is just before breakdown of the nuclear membrane, a spindle structure, the central spindle, appears between the two centrioles (Fig. 31). The centrioles have enlarged slightly during the prophase.

In the metaphase stage (Fig .33) the centrioles are located in the orthodox positions at opposite ends of the spindle. They are enclosed within a small, apparently homogeneous region, the periphery of which is continuous with the slender curved spindle fibres that are attached at their opposite ends to the chromosomes.

In the anaphase (Figs. 34 and 35) the centrioles are slightly smaller but otherwise they are exactly as in the metaphase.

In the early telophase (Fig. 36), just as the nuclear membrane is developing, it can first be clearly seen that the centrioles are doubled, and by late telophase (Figs. 37 and 38) a sphere is reorganized around the two centrioles in each of the sister cells and the cytoplasm has returned to a condition practically like that of the resting cell of Fig. 26.

DISCUSSION

In the observations just described I have given examples of centrioles in a number of vertebrate tissue cells. By other workers these structures have been described in almost every other type of vertebrate and many invertebrate cells. The one outstanding exception of which I am aware is the striated muscle fibre, in which we have no clue to the presence of centrioles except the remarkable suggestion of Wolbach (1928) that in this cell the centrioles increase enormously in number and become incorporated as parts of the myofibrillæ. Personally I feel that this suggestion awaits confirmation and additional evidence before it can be seriously considered. In every case that I have studied the centrioles are two small granules that are closely adjacent to one another but morphologically independent. I agree with Joseph (1903) in being unable to convince myself of any instance where the two are connected by a fibre, the so-called centrodesmus, such as has been frequently described. I am inclined to believe that the apparent demonstration of such a structure has been due in many instances to the difficulty of getting actually clear-cut differentiation of the centrioles after staining with iron hematoxylin, a situation not encountered with the crystal violet method.

In some respects the flagellated cells are of more theoretical interest than any other type of tissue cell. They were first seen by Zimmerman (1898) in the thyroid of elasmobranch fishes (an observation more recently confirmed by Cowdry, 1921), in the uriniferous tubules of the rabbit, and in the human pancreatic duct and seminal vesicle. Meves (1899) observed the presence of a flagellum attached to the distal centriole in cells of the proximal segment of the mesonephric tubule of the salamander. It is Joseph (1903) to whom we are indebted for the most comprehensive studies of these flagellated cells as well as for the only really excellent discussion of their theoretical significance. He described and figured beautifully these cells in the epithelium of the atrium of the adult and of the entire body epidermis of the larva of *Amphioxus*, in the kidney tubules of *Torpedo*, *Salamandra*, and the rabbit, and in parts of the lining of the membranous labyrinth of the ear of mammalian embryos. In nearly every instance Joseph found a thread-like structure running inward from the proximal centriole toward the nucleus, an observation I have been unable to confirm except in one type of cell. The actual function of these flagellated cells is not known. They occur in small number among non-flagellated cells in the pancreatic duct and it is difficult to believe in this instance—or for that matter even in the larval amphibian kidney, where they occur on practically every cell—that such tiny structures could actually be of much consequence in movement of fluid along the tubule. This occurred to Zimmerman and he suggested that the flagella were actually sensory in function, serving to detect the rate of passage of material along the tubule into which they project. In that case they would presumably be non-motile. The manner in which the flagella are curved, however, certainly seems to suggest that they are actively motile.

What data we have on the ontogenetic and phylogenetic distribution of flagellated cells indicates that they are of more frequent occurrence in the lower vertebrates and in the developmental stages. I can offer confirmation of this in the fact that I am unable to find flagella in the adult urodele (*Necturus*) kidney, although here the centrioles are clear enough in their orthodox superficial position. On the whole, these facts urge me to agree with Joseph in his view that these flagella in vertebrate cells are merely vestigial structures reminiscent of a remote ancestral type of animal which had flagellated cells throughout extensive areas of epithelium lining the internal cavities of the body. One is at once reminded of the possibility of some form similar to the sponges, in which the choanocyte is essentially just this type of cell. If one accepts this view the vertebrate flagellated cells assume considerable significance since one might regard them as one of the most primitive vertebrate tissue cells.

A further theoretical significance attaches to the flagellated cells because of the support they offer to the Henneguy-Lenhossék (Henneguy, 1897; von Lenhossék, 1898) view of the centrioles as blepharoplasts from which are developed not only flagella of epithelial cells and the axial filament of the spermatozoön but also all ciliary processes. Joseph has shown examples of cells with several flagella alongside others with a single process each. Such examples strongly suggest how, by further multiplication, a true ciliated cell might arise. The most recent and in many respects the most conclusive contribution to the Henneguy-Lenhossék hypothesis is the work of de Renyi (1924) on the tracheal epithelium of the human fetus. This author figures a very convincing closely graded series of stages in multiplication of the original two centrioles prior to the formation of ciliary processes. In its original form the Henneguy-Lenhossék hypothesis included the corollary that ciliated cells are incapable of mitotic division because of the fact that all the centrioles are, as it were, "employed" as basal bodies attached to cilia. Much of the evidence used to combat the theory has attacked this very point. Various authors (for example, Wallengren, 1905; Erhard, 1910; Saguchi, 1917) have held that observations of mitotic figures in ciliated cells or the demonstration of centrioles that are not blepharoplasts in the non-dividing ciliated cells to a considerable extent invalidate the entire Henneguy-Lenhossék hypothesis. Critical consideration is rather destructive to many of these contradictory data but even if it is granted at its face value I am unable to see why the theory of the centrioles as the only blepharoplast of vertebrate tissues is not equally plausible if merely altered to assume either that one pair of the numerous centrioles produced prior to the development of cilia remains to function as division centers, or even that a blepharoplast centriole is capable of becoming detached from the cell process and going through the orthodox behavior of centrioles at mitosis.

A most important consideration with respect to the centrioles of non-dividing cells is the question of whether they are self-perpetuating cell components. This problem today resolves itself practically into the task of proving first, that the centrioles of the non-dividing cells go to the poles of the spindle at mitosis, and second, that they are bodies persistent from a telophase of a previous division, during which they were formed by division of one of the parent centrioles. In view of the importance of obtaining crucial evidence bearing on this question, it is difficult to understand why there should be very few instances of this sort of work. All the existent positive evidence, however, is in complete accord with the orthodox view that the centrioles reproduce themselves at cell-division. It is, of course, well known that in many instances of spermatocyte development the centriole history, including its multiplication, can be completely demonstrated from an early growth period to the spermatid. Very likely the conclusive character of this demonstration has made it seem that the addition of similar cases in somatic cells, where the task is much more difficult, was unnecessary. The evidence of the capacity for self-perpetuation possessed by centrioles in a case like that of the leucocyte described in Part II is as conclusive as is possible from study of fixed material. Tschassownikow (1914) has also demonstrated with equal clearness the complete centriole cycle in the goblet-cell. This example is particularly satisfactory because the centrioles of the non-dividing goblet-cell have been observed in fresh material by Heiderich. To me it seems that this adds the final touch necessary to make the story completely convincing. Ballowitz (1898) has described an equally good case of centriole continuity in the epithelium of *Salpa.* Heidenhain (1907) has traced out a similar complete centriole history in the mitosis of the erythrocyte of the duck embryo. Very recently Wilson and Huettner (1931) have described complete genetic continuity of the centrioles in the late divisions of the egg of *Drosophila.*

In addition to the complete demonstrations of centriole history enumerated above there are various other suggestive facts in the scanty literature on mitotic phenomena in somatic cells. An instance is the prophase phenomena in division of the pancreatic epithelium (Pollister, 1929). In gland cells, as described on p. 5, the centrioles are located between the nucleus and the lumen end of the cell, nearer the latter. In the pancreas cell this region is filled with prozymogen granules which stain heavily with crystal violet so that it is impossible to determine that the centrioles are actually present in this position. However, by analogy with other gland cells one is reasonably safe in assuming the presence of centrioles inside the mass of prozymogen granules; and this assumption is rendered more likely by the fact that in the early prophase an aster and centriole appear in exactly this region and ultimately give rise to the centrioles and asters at the ends of the spindle. There is some evidence that these centrioles divide during the later stages of mitosis, but I do not wish at this time to stress the later history of the centrioles in the division of pancreatic cells due to certain difficulties in interpretation of the telophase phenomena.[1] It is at any rate significant that the prophase events in the pancreas cell are completely in line with other cases where the complete history of the centrioles is demonstrable.

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[1] A possible suggestion as to how this problem of returning the centriole to its orthodox position in the non-dividing cell may be solved is offered by the work of Poska-Teiss, 1933.

Another interesting case is that of the mitotic phenomena of the smooth muscle cell (Pollister, 1932*b*). The early metaphase spindle is at right angles to the cell axis, apparently foreshadowing a longitudinal splitting of the fibre in the later stages. But the cleavage ultimately occurs in a plane at right angles to the long axis of the fibre because of rotation of the spindle in the later metaphase. The position of origin of the metaphase spindle, which is at first sight somewhat puzzling, is easily understood when it is realized that in the non-dividing cell the centrioles are located midway along the length of the nucleus, a condition brought about by rotation of the chromosome plate and the associated centrioles in the telophase of the last division. Apparently, when the centrioles separate in the prophase to go to opposite sides of the nucleus they migrate the least distance that will bring them into this relationship to the nucleus. This, of course, places them on opposite sides of the nucleus midway along its length, and as a consequence the spindle arising between the centrioles is at right angles to the long axis of the nucleus and of the cell.

In the aggregate it seems to me that this mass of data on the centriole history offers completely adequate support for the view that the animal tissue cells characteristically possess a pair of centrioles which perpetuate themselves at each mitotic division.

Conclusion

In concluding this short discussion of some aspects of centrioles I will briefly summarize what seem to be the essential facts concerning these components of the cytoplasm of vertebrate tissue cells. *The centrioles are minute self-perpetuating bodies characterized by somewhat specific staining reactions but more by their specific location in each type of cell and by their behavior. Centrioles show two general characteristic functions: First they have the capacity of functioning as blepharoplasts at the bases of motile cell processes—flagella or cilia. Second, in the prophase of mitotic cell-division they assume positions that determine the orientation of the spindle—an event that is the initial visible indication of the bipolarity that foreshadows division into two daughter cells.*

LITERATURE CITED

Ballowitz, E., 1898. Zur Kenntnis der Zellsphäre am Salpenepithel. *Arch. f. mikr. Anat. u. Phys. Anat.* Abt.

Bělǎr, K., 1926. Cytologische Grundlage der Vererbung. Berlin.

COWDRY, E. V., 1921. Flagellated Thyroid Cells in the Dogfish (Mustelus canis). *Anat. Rec.*, **22**: 289.

ERHARD, H., 1910. Studien über Flimmerzellen. *Arch. f. Zellf.*, **4**: 309.

FLEMMING, W., 1891. Attraktionssphären und Centralkörper in Gewebszellen und Wanderzellen. *Anat. Anz.*, **6**: 78.

HEIDENHAIN, M., 1907. Plasma und Zelle. Jena.

HEIDERICH, F., 1910. Sichtbare Centrosomen in überlebenden Zellen. *Anat. Anz.*, **36**: 614.

HENNEGUY, L., 1897. Sur les rapports des cils vibratiles avec les centrosomes. *Arch. d'anat. micr.*, **1**: 481.

JOSEPH, H., 1903. Beiträge zur Flimmerzellen- und Centrosomenfrage. *Arb. aus d. Zool. inst. d. Univ. Wien u. d. Zool. Stat. in Triest*, **14**: 1.

VON LENHOSSÉK, M., 1898. Ueber Flimmerzellen. *Verh. d. Anat. Ges.* Kiel.

VON LENHOSSÉK, M., 1899. Das Mikrocentrum der glatten Muskelzellen. *Anat. Anz.*, **16**: 334.

MEVES, F., 1899. Ueber ein Einfluss der Zelltheilung auf den Secretionsvorgang. Festsch. z. 70 Geburtstage v. C. von Kuppfer.

POLLISTER, A. W., 1929. Notes on Cell Division in the Pancreas of the Dogfish. *Anat. Rec.*, **44**: 29.

POLLISTER, A. W., 1932*a*. The Centriole of the Amphibian Leucocyte. *Science*, **75**: 390.

POLLISTER, A. W., 1932*b*. Mitosis in Non-striated Muscle Cells. *Anat. Rec.*, **53**: 11.

POSKA-TEISS, L., 1933. Spermatogonien von Bufo vulgaris Laur. *Zeitschr. f. Zellf. und mikr. Anat.*, **17**: 347.

DE RENYI, G., 1924. Untersuchungen über Flimmerzellen. *Zeitschr. f. Anat.*, **73**: 338.

SAGUCHI, S., 1917. Studies on Ciliated Cells. *Jour. Morph.*, **29**: 217.

VON SMIRNOW, A. E., 1906. Über die Mitochondrien und den Golgischen Bildungen analoge Strukturen in einigen Zellen von Hyacinthus orientalis. *Anat. Hefte*, **32**: 145.

SPULER, A., 1896. Beiträge zur Histologie und Histogenese der Binde- und Stützsubstanz. *Anat. Hefte*, **7**: 115.

TSCHASSOWNIKOW, S., 1914. Über Becher- und Flimmerepithelzellen und ihre Beziehungen zueinander. *Arch. f. mikr. Anat.*, **84**: 150.

VAN BENEDEN, E., 1876. Recherches sur les Dicyémides. *Bull. Acad. Roy. de Belgique*, vol. **41**.

WALLENGREN, H., 1905. Zur Kenntnis der Flimmerzellen. *Zeitschr. f. allg. Physiol.*, **5**: 351.

WILSON, E. B., AND A. F. HUETTNER, 1931. The Central Bodies Again. *Science*, **73**: 47.

WOLBACH, S. B., 1928. Centrioles and the Histogenesis of the Myofibril in Tumors of Striated-Muscle Origin. *Anat. Rec.*, **37**: 255.

ZIMMERMAN, K. W., 1898. Beiträge zur Kenntnis einiger Drüsen und Epithelien. *Arch. f. mikr. Anat.*, **52**: 552.

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